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A NEW, CRYPTIC SPECIES OF *PHYSALAEMUS* (ANURA: LEPTODACTYLIDAE) FROM WESTERN ECUADOR WITH COMMENTS ON THE CALL STRUCTURE OF THE *P. PUSTULOSUS* SPECIES GROUP

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Abstract: We describe a new species of leptodactylid frog of the genus *Physalaemus* from the lowlands of western Ecuador. It belongs to the *P. pustulosus* species group and differs from other group members in its smaller size, skin texture, tadpole characters, and advertisement call. The new species has wide variation in size and color pattern both at the intra- and inter-population levels. This variation matches that observed in *P. montubio* and *P. randi*, and render difficult its diagnosis on the basis of morphological features. A phylogenetic analysis of morphological characters shows that the new species belongs to a clade distributed west of the Andes in Ecuador and northern Peru, sister to (*P. petersi* + *P. pustulosus*). A phylogeny based on mtDNA shows that *P. montubio* and *P. randi* form a clade sister to (*P. coloradorum* + *P.* sp. nov.). The calls of these three species have two components with different acoustic features that arguably match the frequency sensitivity of the ear of conspecific females, in a manner similar to the complex calls of *P. pustulosus*. The new species occurs in two predominantly dry regions, the Lowland Deciduous Costa Forest, and the Lowland Semideciduous Costa Forest.

Key words: Anura; Advertisement call; Ecuador; Leptodactylidae; Morphology; New species; Phylogeny; *Physalaemus guayaco*; *Physalaemus montubio*; *Physalaemus pustulatus*; *Physalaemus pustulosus* group; *Physalaemus randi*; Sensory exploitation hypothesis; Tadpole

Ecuador has one of the highest amphibian species richnesses, with a total of 441 described species (updated from Coloma and Quiguango, 2000–2003). Within Ecuador, the biogeographic regions with the highest regional diversities are the montane forests of the Andean slopes. Conversely, the most depauperate amphibian communities are those from the driest habitats in Ecuador, the Dry Costa Shrub and the Deciduous and Semideciduous Costa forest (S. R. Ron, unpublished; vegetation types as defined in Sierra et al., 1999).

Although covering an area of approximately 33,700 km^2 (Sierra et al., 1999) only 25 amphibian species have been recorded in these dry habitats. At least 19 of those amphibians have significant portions of their distribution ranges in adjacent habitat types, especially the Chocoan Tropical Rainforest. The remaining species have distributions restricted (or nearly so) to dry habitats and are therefore unique elements of the amphibian fauna. These species are *Ceratophrys stolzmanni, Leptodactylus labrosus, Physalaemus pustulatus, Colostethus infraguttatus, C. elachyhistus,* and *Rana bwana*.

Surveys carried out in 2002 and 2003 at the Deciduous and Semideciduous Costa forest have resulted in additions to this short list. Two species of *Physalaemus* belonging to the *P. pustulosus* species group have been described recently (Ron et al., 2004). Herein, we describe an additional species of the same group from the lowlands of western Ecuador. During fieldwork in 2002, specimens of the new species initially were misidentified as *P. montubio* because of similarities of their calls and external morphology. However, analyses of mitochondrial DNA and a careful examination of the morphology of adults made evident the distinctiveness of this new species from other members of the *P. pustulosus* group.

Methods

Morphological terminology and abbreviations follow Lynch and Duellman (1997) for adults and Altig and McDiarmid (1999) for tadpoles. Osteological characters used in the diagnosis were examined in clear-and-stained

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(C & S) specimens and are defined in Cannatella and Duellman (1984) and Cannatella et al. (1998). Sex was determined by the presence of nuptial pads, vocal sac folds, and/or by gonadal inspection. Tadpoles were staged according to Gosner (1960). Snout–vent length is abbreviated as SVL throughout. Examined specimens (listed in the type-series and Appendix I) are housed in the American Museum of Natural History (AMNH), California Academy of sciences (CAS), Museo de Zoología de la Pontificia Universidad Católica del Ecuador (QCAZ), Museum of Comparative Zoology Harvard University (MCZ), National Museum of Natural History (NMNH), and Natural History Museum University of Kansas (KU). Geographic coordinates for specimens collected in 2002 and 2003 were measured with a Garmin™ 12CX GPS receiver, based on the geodetic datum WGS 84.

Sound recordings were made with a Sennheiser™ ME-67 directional microphone and a Sony™ WM-D6C analog tape recorder. Calls were analyzed using Canary™ 1.2.1 software (Charif et al., 1995) at a sampling frequency of 22.1 kHz and a frequency grid resolution FTP = 8192. We measured eight call parameters: (1) call duration (time from beginning to end of call); (2) rise time (time from the beginning of the call to the point of maximum amplitude); (3) interval between calls (time between the end of one call and the beginning of the next call); (4) duration of the first component (time from beginning of the first pulse to the end of the last pulse of the first component of the call, expressed as percentage of call length); (5) call repetition rate (number of calls/min); (6) fundamental frequency of the first component (frequency of the first harmonic along the duration of the first component of the call); (7) dominant frequency of the first component (frequency with the most energy along the duration of the first component of the call); and (8) fundamental frequency of the second component (frequency of the first harmonic along the duration of the second component of the call). Original recordings are deposited in the audio archive of the QCAZ and the Texas Natural History Collection of the Texas Memorial Museum (University of Texas at Austin).

Shape and size of parotoid glands and flank glands were determined by making a middorsal incision to reflect the skin, which allowed for inspection of its internal surface. Length of the parotoid gland and flank gland were measured from the anterior to the posterior edge. To avoid specimen deterioration, we measured gland length of only one third of the available specimens. All well-preserved specimens (Simmons, 2002) were measured for the following morphometric variables: (1) SVL; (2) head length; (3) head width; (4) eye–nostril distance; (5) femur length; (6) tibia length; (7) arm length; and (8) dorsum width. Measurements were made according to the methodology described in Duellman (1970) and Ron et al. (2004) with Fowler digital calipers (nearest 0.01 mm) from specimens fixed in 10% formalin and preserved in 70% ethanol. Because of small sample sizes for females, most morphometric comparisons were made only among adult males. Tadpole measurements were taken from digital images using the measurement tool of Adobe® Photoshop® 7.0 (Adobe Systems Incorporated, 2002).

Multivariate analyses were used to assess the degree of morphometric differentiation among species. To remove the effect of covariation with SVL, Principal Components Analysis (PCA) and Discriminant Function Analysis (DFA) were applied to the residuals of the linear regressions between the seven measured variables and SVL (Vitt et al., 2000). For the PCA, only components with eigenvalues $>$ 1 were retained. The species included in the analyses are (number of specimens in parentheses): *P.* sp. nov. (55); *P. montubio* (101); *P. pustulatus* (24); and *P. randi* (35). StatView® 5.01 (SAS Institute Inc., 1998) was used to perform the PCA; JMP® 4.0 (SAS Institute Inc., 2000) was used to perform the DFA.

Climate parameters for localities were estimated from digital climate maps compiled by C. Graham, using ArcMap 8.2 with the SpatialAnalyst extension (ESRI, 2001).

Phylogenetic Analyses

Morphology.—We expanded the phylogenetic analysis made by Cannatella et al. (1998) to include the recently described species (herein and *P. montubio* from Ron et al., 2004). In the Cannatella et al. (1998) phylogeny, *Physalaemus randi* and *P. pustulatus* are mistakenly referred as "*P. pustulatus*" and "*P.* sp. C", respectively. States of characters 1–12 were scored following Cannatella et al. (1998), except that the flank gland was scored as absent or present. The only addition to the matrix was character 13 (SVL); it was scored by step-matrix gap-weighting (scaled to 999, Appendix II; Wiens, 2001). Between-character scaling was used to give the same maximum length to binary characters (qualitative; 1–12) and quantitative characters (SVL; Wiens, 2001); nonstep-matrix characters have a weight of 999. *Physalaemus enesefae* and *P. ephippifer* were used as outgroups. Parsimony analyses were performed with PAUP* 4.08 (Swofford, 2000) using the exhaustive search algorithm. The data matrix is shown in Appendix II.

Mitochondrial DNA sequences.—We sampled 11 populations of *Physalaemus* from western Ecuador, belonging to five species. The primary purpose of the molecular analyses presented here is to provide evidence of the distinctiveness of *P. guayaco* with respect to its more closely related species. A more comprehensive phylogeny including all species of the species group will be published elsewhere. DNA was extracted from liver tissue samples stored in 95% ethanol. We analyzed 2409 bases of mitochondrial DNA genes12S rRNA, valine-tRNA, and 16S rRNA. Methods for DNA extraction, amplification, and sequencing followed the protocol of Santos et al. (2003). Preliminary alignment was done with CLUSTAL X 1.8 (Thompson et al., 1997) over a matrix containing *P. pustulosus, P. freibergi, P. petersi*, and 25 species of *Physalaemus* outside the *P. pustulosus* species group. The ambiguously-aligned regions were adjusted by eye to produce a parsimonious alignment (i.e., informative sites minimized). Parsimony and maximum likelihood (ML) analyses were performed with PAUP* 4.08 (Swofford, 2000). The most parsimonious tree was found with a branch-and-bound search and the ML tree with heuristic searches using TBR branch swapping, random addition sequence of taxa and 10 replicates per search. Characters were unordered and equally weighted for parsimony analyses. Maximum likelihood searches were performed under six nested models of nucleotide substitution. The best fitting model was GTR + Γ according to an ML ratio test (Huelsenbeck and Crandall, 1997). Parsimony and ML analyses (all models) yielded trees with identical branching pattern. *Physalaemus pustulatus* was used as an outgroup based on its position in the Cannatella et al. (1998) "combined" phylogeny (referred to as "*Physalaemus* sp. C" in that work). The basal position of *P. pustulatus* within the clade of *Physalaemus* distributed in northwestern South America is corroborated by the morphology-based phylogeny (Fig. 1) and additional unpublished mtDNA sequence data by DCC, including *P. pustulosus, P. freibergi, P. petersi*, and 25 species of *Physalaemus* outside the *P. pustulosus* species group. Locality of specimens and GenBank accession numbers are shown in Appendix III. In both the morphology and DNA-based phylogenies, clade support was estimated from non-parametric bootstrapping with heuristic searches of 1000 replicate data sets.

Systematic Account

Physalaemus **guayaco** *sp. nov*

Holotype.—(Fig. 2) QCAZ 23521 (field no. PUCE 8430), adult male from Ecuador, Provincia del Guayas, Cerro Masvale (private reserve at 2.394° S, 79.642° W), 92 m, collected by M. R. Bustamante, I. G. Tapia, and S. R. Ron on 23 March 2003.

Paratopotypes.—QCAZ 19381–82, 19561–62, 19751, adult males, collected by D. C. Cannatella, L. A. Coloma, A. Holloway, and S. R. Ron on 20 February 2002; QCAZ 23505–12, 23514–15, 23516 (C & S), 23517, 23519–21, adult males, 23518 (C & S), adult female, collected by M. R. Bustamante, I. G. Tapia, and S. R. Ron on 23 March 2003.

Paratypes.—Ecuador: Provincia del Guayas: 20 km E of Durán on the road to Milagro (2.022° S, 79.690° W), 32 m, QCAZ 23445, adult male; 11 km N Cerro Masvale, on the road to Virgen de Fátima (2.300° S, 79.639° W), 40 m, QCAZ 23522, 23525–27, 23532–37, 23541, 23559–570, 23571 (C & S), 23572, 23573 (C & S), 23574, 23575 (C & S), 23576–78, adult males, 23531, adult female; 15 km S Naranjal, on road to Machala (2.766° S, 79.692° W), 74 m, QCAZ 23650, 23652–53, 23655–56, adult males, 23654, adult female, 23651 juvenile. Collected by M. R. Bustamante, I. G. Tapia, and S. R. Ron between 20 and 25 March 2003.

Diagnosis.—A member of the *P. pustulosus* group, sensu Cannatella and Duellman (1984) and Cannatella et al. (1998). The assignment to the *P. pustulosus* group is based on the presence of four synapomorphies (Cannatella et al., 1998): (1) presence of flank glands; (2) presence of parotoid glands; (3) warty skin; and (4) dentigerous process of the vomer thin and spikelike.

Physalaemus guayaco (Fig. 2) is characterized by: (1) mean SVL 16.85 mm in males (range 15.45–19.38; *n* = 55) 18.67 mm in females (range 16.77–20.98; *n* = 3); (2) skin on dorsum bearing scattered tubercles; (3) snout varying between truncate and subacuminate in dorsal view and round in lateral view; (4) vomerine teeth and odontophores absent; (5) maxillary and premaxillary teeth present; (6) parotoid glands present, mean length = 1.90 mm (*n* = 23; SD = 0.50; 6.3–15.6% of SVL); (7) flank glands present, mean length = 3.93 mm (*n* = 23; SD = 1.14; 10.3–32.1% of SVL); (8) tarsal tubercle absent; (9) nuptial pads present; (10) Finger I shorter than II; (11) tympanic annulus evident, concealed dorsally, tympanic membrane not tuberculate.

Physalaemus guayaco is smaller than *P. petersi, P. pustulosus,* and *P. pustulatus* (non overlapping SVL; Cannatella and Duellman, 1984; Ron et al., 2004) and has a different advertisement call (Ron et al., 2004; Ryan and Rand, 2001; Fig. 3). The absence of a tarsal tubercle and the presence of teeth in the maxilla and premaxilla further distinguish it from *P. petersi* and *P. pustulosus*. The tadpole of *P. guayaco* differs from that of *P. petersi* in lacking paired elliptical paravertebral glands and in dorsal coloration (Fig. 4). *Physalaemus coloradorum* has a longer advertisement call without well-defined pulses at the beginning (Ryan and Rand, 2001), and bigger dorsal tubercles, some coalescing into ridges (tubercles are more scattered and never coalesce into

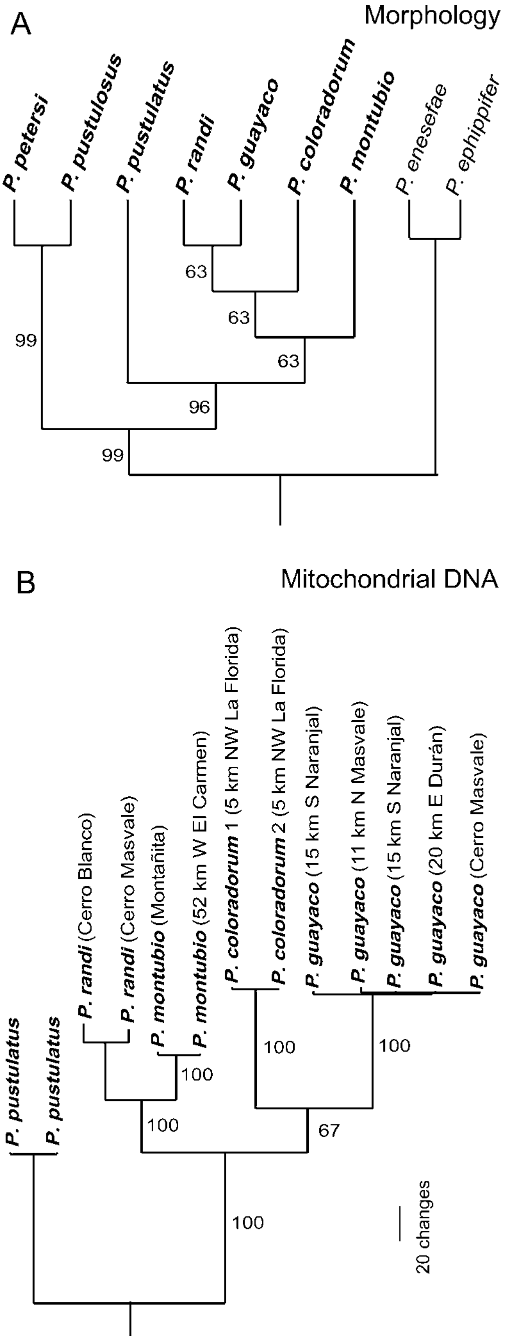

FIG. 1.—(A) Single, most parsimonious tree of the phylogenetic relationships of the *Physalaemus pustulosus* species group (boldface taxa) based on 13 morphological characters (tree length = 13.31 [raw TL = 13299], CI = 0.976, consistency index [excluding uninformative characters] = 0.975, RI = 0.543). Raw tree length is divided by 999 because of the use of a step-matrix to code one morphometric character (scaled to 999; Appendix II for

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details). (B) Single, most parsimonious rooted phylogram (length of branches proportional to the amount of estimated change for that lineage) based on 2409 bases of mtDNA genes12S rRNA, valine-tRNA, and 16S rRNA (TL = 455, consistency index = 0.888, CI [excluding uninformative characters] = 0.884, RI = 0.935). In (A) and (B), numbers next to internal branches are nonparametric bootstrap support values (from 1000 iterations). Maximum likelihood analysis (best-fit model GTR+Γ) yielded a single tree (not shown) with identical topology to that from parsimony.

FIG. 2.—Dorsolateral and ventral views of *Physalaemus guayaco* from 15 km S Naranjal, Provincia del Guayas, Ecuador: (A) QCAZ 23531, adult female, SVL = 18.26; (B) 23510, adult male, SVL = 16.13.

ridges in *P. guayaco*; Fig. 4). *Physalaemus coloradorum* further differs from *P. guayaco* in having a vertical loreal region (oblique in *P. guayaco*). *Physalaemus guayaco* is most similar to *P. montubio* and *P. randi*; the feet of both species have less extensive lateral fringes and basal webbing than *P. guayaco*. *Physalaemus guayaco* has a shorter call (Fig. 3; Table 1; Ron et al., 2004) and smaller and less abundant dorsal tubercles than *P. randi* (Fig. 5). *Physalaemus montubio* is larger than *P. guayaco* (male mean SVL = 20.6 mm [*n* = 117; SD = 1.16; specimens from eight populations] and 16.85 [*n* = 55; SD = 0.99; from three populations], respectively; *t* = 20.66; df = 170; *P* < 0.0001; Fig. 6). Size differences are not significant between the population of *P. montubio* with the lowest average SVL (52 km W El Carmen on the road to Pedernales, Provincia de Manabí) and that of *P. guayaco* with the highest average SVL (15 km S Naranjal, Provincia del Guayas; *t* = 0.79;

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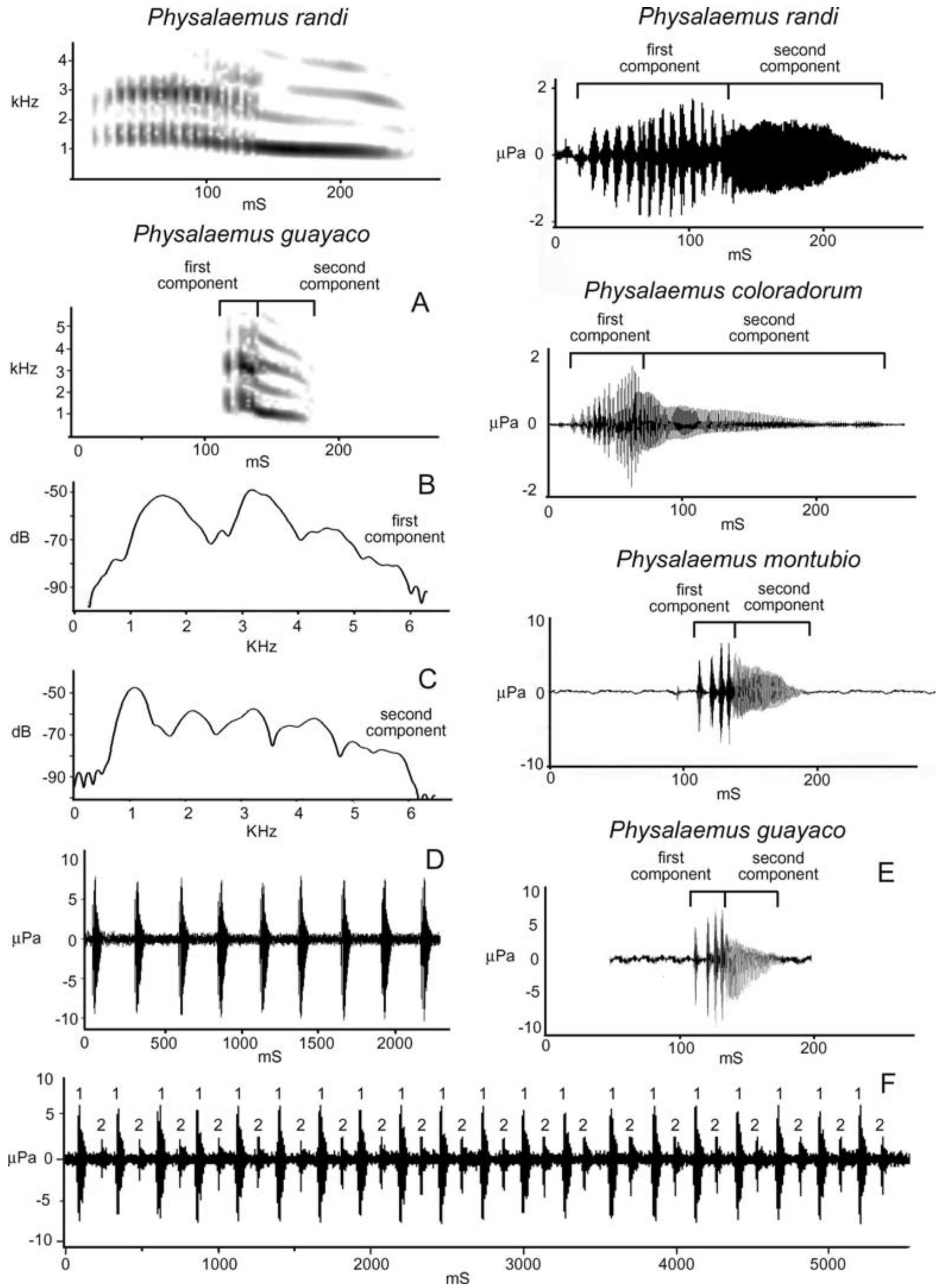
FIG. 3.—Calls of *Physalaemus* from western Ecuador. Letters are for *P. guayaco* (A–E from QCAZ 19562; F from QCAZ 23508 and uncollected male) recorded at Cerro Masvale, Provincia del Guayas, Ecuador. (A) Sonogram of single call, (B) power spectrum of the first call component, (C) power spectrum of the second call component, (D) oscillogram of series of calls, (E) oscillogram of single call, (F) oscillogram of the antiphonal calls of two males. Calls from QCAZ



23508 are marked "1"; calls from a noncollected male are marked "2". The first component is a series of amplitude-modulated pulses; the second component is a whine-like note, its first harmonic is a nearly pure tone. There is a switch in the dominant frequency between the first and the second component. The first and second components also are evident in the oscillograms of the advertisement calls of *P. randi* (QCAZ 19752, Cerro Masvale), *P. coloradorum* (QCAZ 19412, 5 km NW La Florida, Provincia del Pichincha), *P. montubio* (QCAZ 23214, San Vicente, Provincia de Manabí) shown in the right column.

df = 8; *P* = 0.45). However, these populations are 630 km apart, at the opposite latitudinal extremes of their species distribution ranges, and therefore are unlikely to represent part of a size gradient within a single species (Figs. 6, 7). Calls of *P. montubio* resemble those of *P. guayaco* except for a small difference in frequency: average fundamental frequency for the first component of the call = 1.475 kHz in *P. montubio* (SD = 104.8; 21 males, 7 populations) and 1.576 kHz in *P. guayaco* (SD = 0.153; Table 1). Differences are close to significant (*t* = 1.782; df = 24; *P* = 0.087), although they may be explained by significant differences in SVL between both samples (*P. montubio* mean SVL = 20.06 mm [SD = 1.35]; *P. guayaco* = mean SVL 17.67 mm [SD = 1.53]; *t* = 3.47; df = 24; *P* = 0.002). Phylogenetic analysis of morphological characters and 2374 bases of 12S and 16S rRNA genes of mtDNA show unambiguously that, despite the similarity of their advertisement calls and external morphology, *P. guayaco* and *P. montubio* are not sister species (see Discussion).

Description of holotype.—Adult male, 16.02 mm SVL, tibia length 7.55 mm, femur length 7.55 mm, arm length 3.69 mm, head length 5.61 mm, head width 5.27 mm, eye–nostril distance 1.81 mm, dorsum width 5.91, head wider than body except in scapular region; diameter of eye twice diameter of tympanic annulus; tympanic membrane and tympanic annulus barely evident, hidden dorsally; tympanic annulus ovoid, longer dorsoventrally; tubercles absent from tympanic membrane or annulus; supratympanic fold absent; head slightly elevated between orbits and flat in intercanthal region; snout rounded in profile and truncated in dorsal view; nostrils slightly elevated, internarial region concave; canthus rostralis rounded; loreal region convex with shallow groove extending from posterior border of nostril to posteroventral border of orbit.

Fingers without expanded discs; nuptial pad present, keratinized, brown, divided in two portions, one covering posterior half of thenar tubercle, other covering base of Finger I. Base of palmar and thenar tubercles ovoid; palmar tubercle less prominent than thenar tubercle; subarticular tubercles with round base, all conical except for round and low second (distal) subarticular tubercle on Finger III; second subarticular tubercle on Finger IV absent; few supernumerary palmar tubercles present. Webbing between fingers absent; relative lengths of adpressed fingers III > IV > II > I. Toes without expanded discs; base of

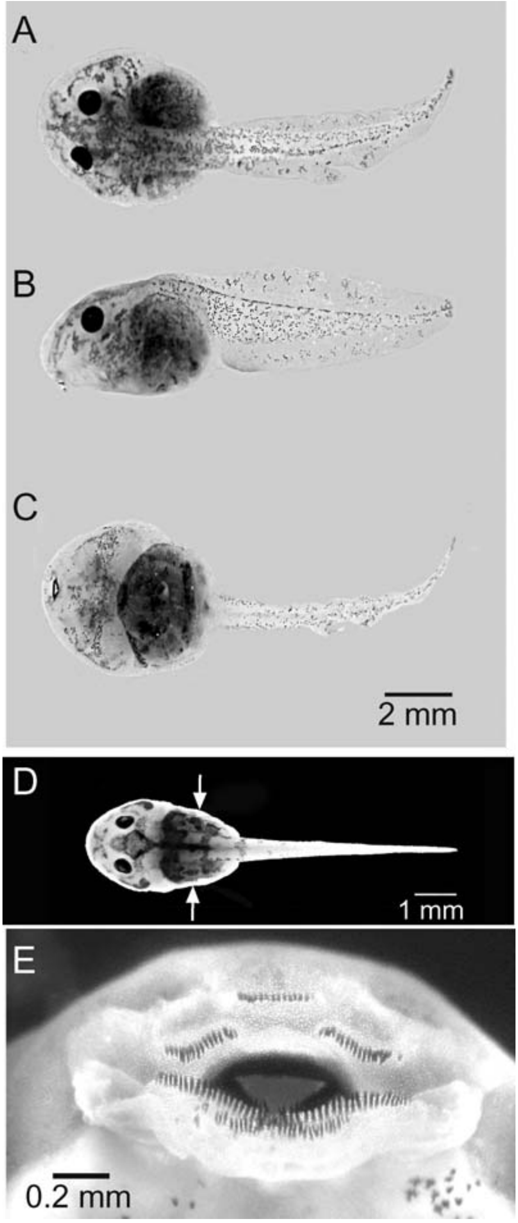


FIG. 4.—Tadpole of *Physalaemus guayaco*, QCAZ 24006A: (A) dorsal, (B) lateral, and (C) ventral views. (D) Tadpole of *Physalaemus petersi* (Stage 35, QCAZ 18282); arrows point to paravertebral glands. (E) Mouth parts of the tadpole of *P. guayaco*, QCAZ 24006B.

TABLE 1.—Call parameters of *Physalaemus guayaco* (ranges in parentheses). The call of *P. guayaco* consists of one note with amplitude-modulated pulses (first component) followed by a "whine" like note with a frequency sweep (second component). Specimen catalog numbers at the Museo de Zoología de la Pontificia Universidad Católica del Ecuador (QCAZ) are shown. Sample sizes are number of calls. See text for details.

Specimen	Mean call duration (ms)	Call repetition rate (calls/min)	Mean interval between calls (ms)	Rise time of the first component (ms)	Mean dominant frequency of the first component (kHz)	Mean fundamental frequency of the first component (kHz)	Mean fundamental frequency of the second component (kHz)
QCAZ 19561	68.2	221.2	203	15.1	1.372	1.372	1.151
$n = 18$	(56.7–76.0)		(161.9–405.1)	(9.1–20.8)	(1.281–1.491)	(1.281–1.491)	(1.125–1.179)
QCAZ 19562	70.6	220.9	201	17.7	3.200	1.577	1.079
$n = 10$	(67.5–72.8)		(188–224)	(16.5–18.8)	(3.149–3.273)	(1.394–1.679)	(1.060–1.098)
QCAZ 23510	52.8	259.6	178.3	14.3	3.237	1.789	1.066
$n = 10$	(49.1–54.9)		(154.1–246.6)	(8.5–15.6)	(3.168–3.341)	(1.744–1.880)	(1.064–1.097)
QCAZ 23512	65.3	259.1	166.3	19.0	3.359	1.511	1.091
$n = 10$	(61.9–68.8)		(153.7–188.1)	(10.4–23.5)	(3.274–3.442)	(1.472–1.703)	(1.070–1.120)
QCAZ 23652	69.4	259.9	190.5	17.9	3.237	1.631	1.048
$n = 15$	(64.6–74.9)		(150.6–270.5)	(10.5–24.4)	(3.004–3.325)	(1.634–1.907)	(1.007–1.084)

inner metatarsal tubercle ovoid, larger than ovoid base of outer metatarsal tubercle; inner metatarsal tubercle more prominent than outer metatarsal tubercle; subarticular tubercles with round base, all conical except for subconical distal subarticular tubercles of Finger IV and V; sparse and minute conical plantar supernumerary tubercles; tarsal tubercle absent; lateral fringes on toes; fringes converge at the base of adjacent toes forming basal webbing; relative lengths of adpressed toes IV > III > V > II > I.

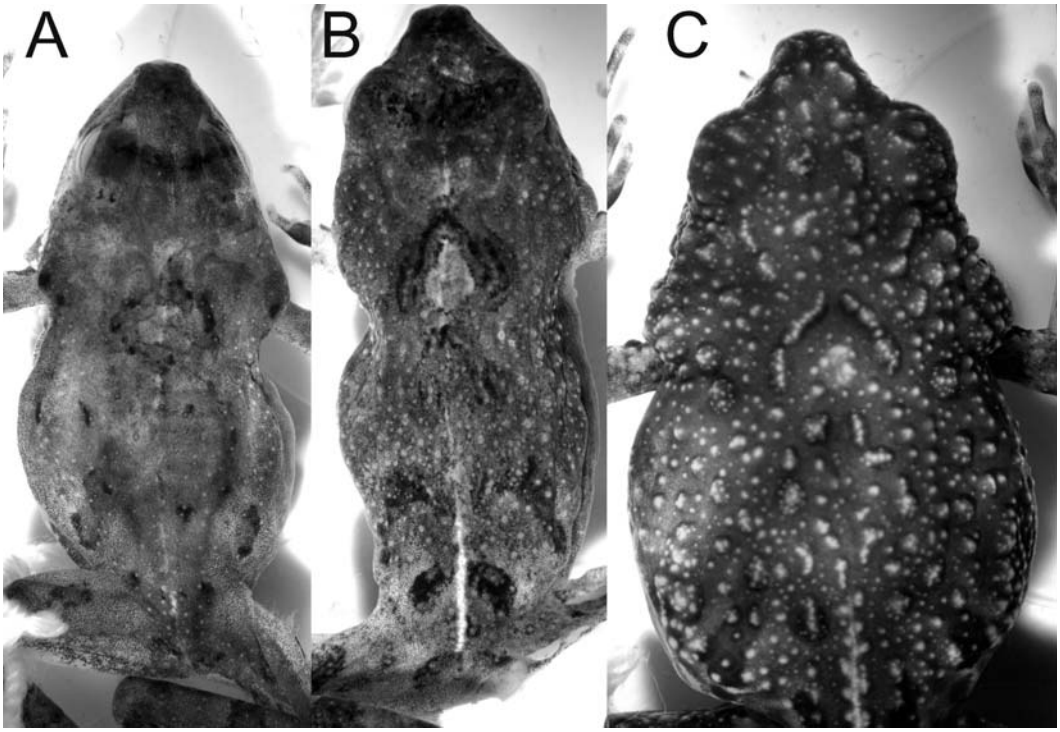


FIG. 5.—Dorsal photographs of adult *Physalaemus guayaco* (A; QCAZ 23510), *P. randi* (B; QCAZ 23579), and *P. coloradorum* (C; QCAZ 2975) showing differences in skin texture. Note that *P. guayaco* has fewer and smaller dorsal tubercles.

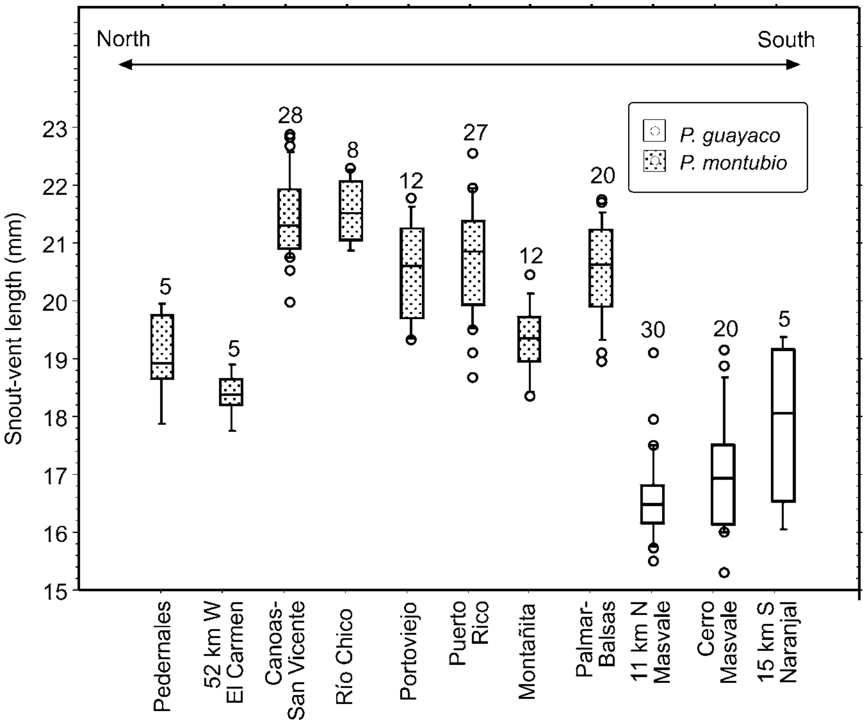


FIG. 6.—Box plot of the snout–vent length of males from eight populations of *P. montubio* (117 specimens) and three populations of *P. guayaco* (55 specimens) from western Ecuador. (See Fig. 8 for locality map). Sample sizes for each locality are shown on top of the box. Sequence of localities from left to right matches their relative geographic position from north to south.

Skin on dorsum bearing minute, round to subconical tubercles, widely scattered anteriorly, some arranged in longitudinal rows; skin on venter smooth. Tongue longer than wide; vomerine teeth and odontophores absent. Maxillary and premaxillary teeth present. Vocal slits present, parallel to margins of mandible. Deflated vocal sac forming folds on gular region, extending posteriorly to proximal end of arm.

Color of holotype in preservative.—Dorsum grayish brown with darker hue on the head, particularly above and between orbits and anterior half of snout; dark gray longitudinal bands weakly defined, running on each side of dorsum, parallel to middorsal axis from scapular to sacral region; faint light gray middorsal line from snout tip to vent, interrupted on intercanthal and interscapular regions; dorsal tubercles light gray; dorsal surfaces of forearms and hind limbs light gray with dark gray marks on forearms and elbows. Venter cream yellowish without marks along posterior one-third of SVL; scattered dark-gray minute spots on chest, some aggregated on dark blotches; minute dark gray spots abundant on ventral surfaces of head except for weakly defined irregular immaculate blotches along jaw margins; ventral surfaces of hind limbs and arms cream yellowish, becoming light gray towards outer and inner edges, some dark blotches present; outer half of ventral surfaces of forearms dark gray; sides of head gray with a light gray area below the orbit and tympanum, extending posteriorly above deflated vocal folds; flanks dark gray dorsally, light gray ventrally; gray appearance on ventral and lateral surfaces of head, limbs, and flanks, results from composite effect of numerous minute melanophores.

Color in life.—(QCAZ 23506, adult male) dorsum grayish-brown with dark marks; subocular bar cream; flanks gray; venter reddish-cream posteriorly; anterior half of venter cream with gray blotches; ventral surfaces of head dark gray with cream midventral stripe

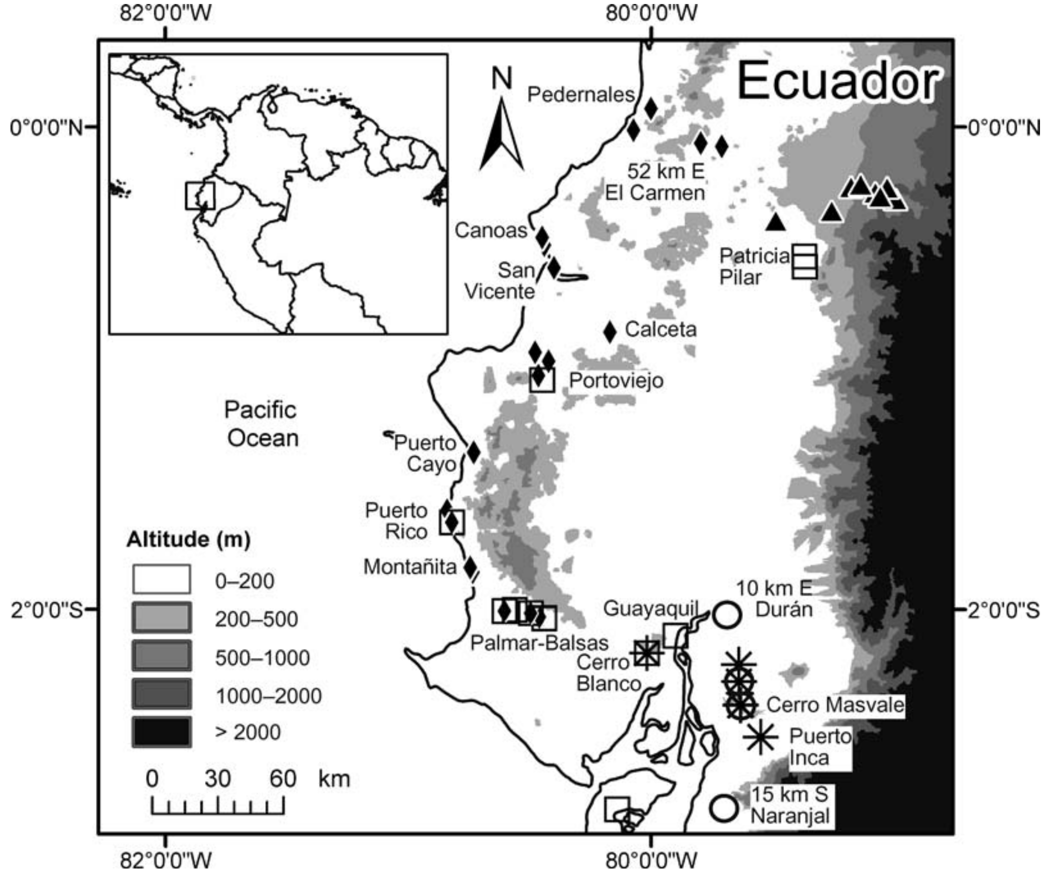


FIG. 7.—Known records of *Physalaemus coloradorum* (triangles), *P. guayaco* (circles), *P. montubio* (diamond), *P. pustulatus* (squares), and *P. randi* (asterisks). Locality data are based on specimens deposited in American Museum of Natural History, California Academy of Sciences, Museo de Zoología de la Pontificia Universidad Católica del Ecuador, Museum of Comparative Zoology Harvard University, National Museum of Natural History, and Natural History Museum University of Kansas (Appendix 1).

from jaw tip to scapular region; thighs salmon red ventrally; iris bronze (S. Ron, field notes).

Etymology.—The specific name *guayaco* is a noun in apposition, in reference to the inhabitants of Guayaquil and Provincia del Guayas, Ecuador.

Variation.—Variation in the dorsal coloration of preserved specimens is extensive (Fig. 8). The background dorsal coloration varies from light gray (QCAZ 23655) to dark gray (QCAZ 23527, 23532). Irregular dark marks may be present in diverse patterns (Fig. 8). The mid-dorsal line varies between well-defined and continuous (from snout tip to vent; QCAZ 23531, 23533, 23652, 23654; Fig. 8), to weakly-defined, restricted to the posterior one-third of the sacral region (QCAZ 23653, 23574; Fig. 8), or absent (QCAZ 23537, 23534; Fig. 8); the line is enclosed within a longitudinal light gray band of irregular width in QCAZ 23656, and 23654 (Fig. 8). In 31 specimens from the type series (e.g., QCAZ 23534, 23561, 23574, 23576; Fig. 8) there is a lighter area in the scapular region. There is also variation in the abundance and arrangement of tubercles (all lighter than the background).

The ventral surfaces of all preserved specimens have a cream to yellowish-cream background with light gray (QCAZ 23564, 23568) to dark gray markings (QCAZ 23653, 23531). Marks may be restricted to the head (darker on folded vocal sacs; QCAZ 23650, 23653) or also present over the entire venter (less abundant posteriorly, QCAZ 23566). Variation between the extremes is continuous. In a few specimens, the ventral marks are arranged in well-defined large spots (QCAZ 23512, 23655). More frequently, these marks form diffuse speckled patterns (e.g., QCAZ 23525, 23560). A midventral cream stripe, extending from near the tip of the snout to the chest, is present in QCAZ 23537. The stripe can be restricted to the gular region (QCAZ 23522) or absent (QCAZ 23569). The arrangement of dark spots and tubercles on the ventral surfaces of the feet and hands of QCAZ 23515 is shown in Fig. 9.

Head shape varies continuously from truncate (QCAZ 23563) to subacuminate (QCAZ 23564, 23566). Lateral head coloration varies between light gray and dark gray. The light area below the eye and tympanum is cream in most specimens (e.g., QCAZ 23567). It may be interrupted by a dark bar below the orbit (QCAZ 23563, 23576) or restricted to a thin longitudinal stripe bordering the orbit and tympanum (QCAZ 23655). There is a dark-gray labial bar below the loreal region in QCAZ 23564 and 23652.

The following morphometric data pertain only to adults. In the type series, the largest male has a SVL of 19.38 mm, and the largest female 20.98 mm; mean male SVL = 16.85 mm (n = 55; SD = 0.99), mean female SVL = 18.67 mm (n = 3; SD = 2.13). The small sample size of females prevents analysis of size dimorphism; however, the available data suggest that females are larger than males. The only female from 11 km N Cerro Masvale (SVL = 18.26 mm; QCAZ 23531) is larger than 96.7% of the males of the same locality (n = 30); the only female from 15 km S Naranjal (SVL = 20.98; QCAZ 23654) is larger than all the males (n = 5); the only female from Cerro Masvale (SVL = 16.77; QCAZ 23518) is larger than 40% of the males (n = 20).

SVL is not significantly different between males of Cerro Masvale and those from 11 km N Cerro Masvale (t = 1.80; df = 48; P = 0.08), or from Cerro Masvale and those from 15 km S Naranjal (t = 4.48; df = 23; P = 0.15). SVL was significantly different between specimens from 11 km N Cerro Masvale and those from 15 km S Naranjal (t = 3.01; df = 33, P = 0.005). Descriptive statistics of morphometric measurements for the three populations are given in Table 2.

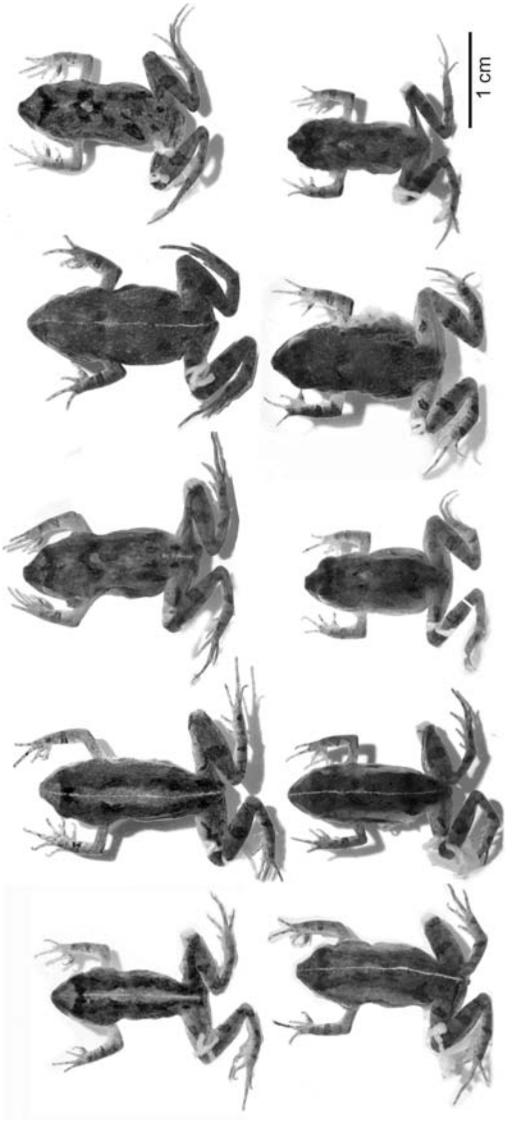


FIG. 8.—Dorsal views of adult *Physalaemus guayaco* showing variation in dorsal patterns. Left to right, upper row: QCAZ 23656 (male), QCAZ 23654 (female), QCAZ 23574 (male), QCAZ 23445 (male), QCAZ 23576 (male); lower row: QCAZ 23652 (male), QCAZ 23531 (female), QCAZ 23561 (male), QCAZ 23557 (male), QCAZ 23534 (male). All from Provincia del Guayas, Ecuador. (See Appendix 1 for locality data).

All morphometric variables shown in Table 2 show a significant positive relation with SVL (simple regressions for males; all ANOVA's $P < 0.005$; df = 52). The relation also is

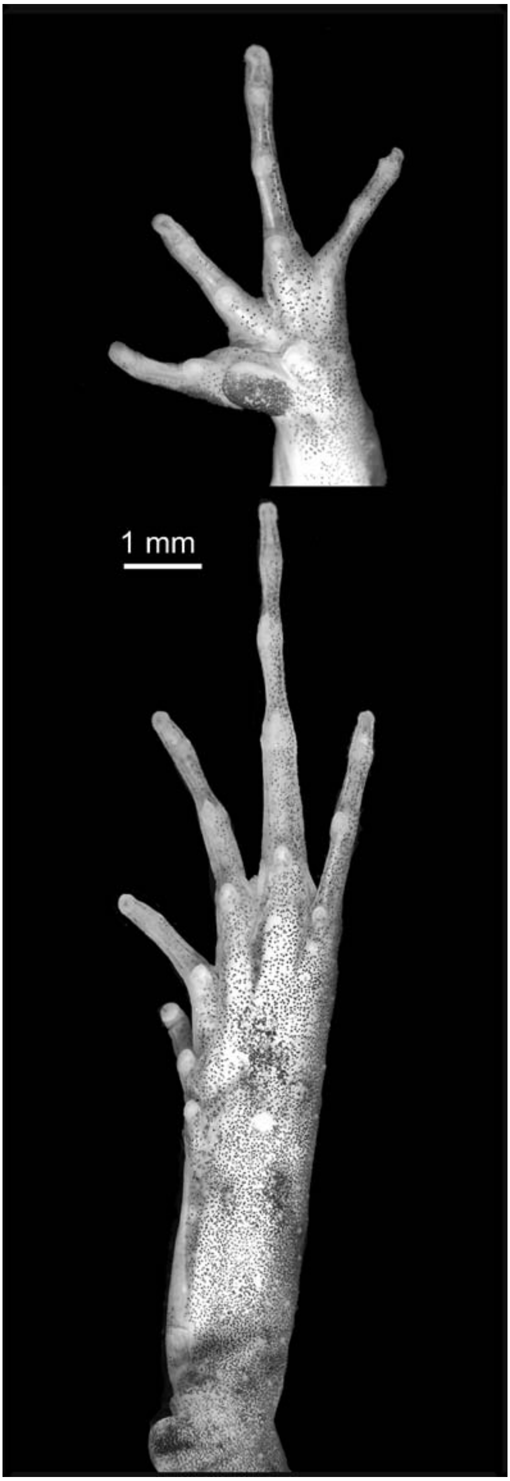


FIG. 9.—Ventral views of the left hand and foot of the paratopotype of *Physalaemus guayaco*, QCAZ 23515 (adult male from Cerro Masvale, Ecuador; SVL = 17.45).

significant between SVL and flank gland length (F = 5.71; df = 21; P = 0.03) and between SVL and parotoid gland length (F = 6.64; df = 21; P = 0.02; specimens from Cerro Masvale and 11 km N Cerro Masvale combined). Flank gland length is correlated with parotoid-flank length (F = 15.76; df = 21; $P <$ 0.001). Total gland length (flank + parotoid) varies between 16.6% of SVL (QCAZ 23567 from 11 km N Cerro Masvale) and 45.6% of SVL (QCAZ 23516 from Cerro Masvale). In all specimens, parotoid and flank glands are separated from each other.

Specimens from 11 km N Cerro Masvale have proportionally shorter flank and parotoid glands than those from Cerro Masvale (Mann-Whitney's U for flank gland length/SVL = 2; $P < 0.0001$; U for parotoid gland length/SVL = 21; P = 0.010; 10 specimens from 11 km N Cerro Masvale and 12 from Cerro Masvale, all males).

Three morphometric variables show proportional differences among populations: (1) dorsum width (Kruskal-Wallis H for dorsum width/SVL = 10.84; P = 0.004); (2) arm length (Kruskal-Wallis H for arm length/SVL = 8.80; P = 0.01); and (3) head width (Kruskal-Wallis H for head width/SVL = 7.58; P = 0.02). Other variables listed in Table 2 do not show significant statistical differences (all Kruskal-Wallis, $P > 0.09$).

Tadpoles.—The following description is based on a lot (QCAZ 24006A–D) of four larvae in Stages 28 (A), 29 (B–C), and 31 (D). Tadpoles were raised in laboratory conditions from a clutch of eggs, that were laid in captivity by female QCAZ 23654, collected on 15 km S Naranjal (Provincia del Guayas) by S. R. Ron, M. R. Bustamante, and I. Tapia on 23 March 2003 at 2100 h. Tadpoles were preserved in formalin 10% on 25 March, 30 April, and 6 May 2003.

These larvae belong to the exotrophic, lentic, bentic ecomomorphological guild as defined by McDiarmid and Altig (1999). Morphometric data are provided in Table 3. A Stage 28 tadpole shows, in dorsal view (Fig. 4), an elliptical body, widest between eye and spiracle, with a rounded snout. Eyes are relatively large (BL about 7.5 times larger than ED), separated by a distance equal to about 1.9 times the internarial distance, directed and positioned dorsolaterally, not visi-

TABLE 2.—Descriptive statistics for morphometric measurements of male *Physalaemus guayaco* from three localities in Provincia del Guayas, Ecuador. Mean ± SD is given with range below. Bold figures are combined for males of all populations. Abbreviations are: SVL = snout–vent length; DW = dorsum width; TL = tibia length; FL = femur length; AL = arm length; HL = head length; HW = head width; EN = eye-nostril distance. All measurements are in mm.

	SVL	DW	TL	FL	AL	HL	HW	EN
P. guayaco								
(*n* = 55)	**16.85 ± 0.99**	**6.20 ± 0.39**	**7.86 ± 0.42**	**7.56 ± 0.51**	**3.9 ± 0.22**	**5.79 ± 0.28**	**5.51 ± 0.32**	**1.83 ± 0.20**
Cerro Masvale	17.02 ± 1.01	6.37 ± 0.23	7.95 ± 0.40	7.72 ± 0.50	3.85 ± 0.24	5.76 ± 0.32	5.57 ± 0.34	1.84 ± 0.19
(*n* = 20)	15.31–19.15	5.91–6.83	7.46–8.91	6.96–8.92	3.41–4.36	5.32–6.41	5.08–6.41	1.54–2.18
11 km N Cerro								
Masvale	16.57 ± 0.75	6.04 ± 0.36	7.74 ± 0.36	7.37 ± 0.46	3.90 ± 0.17	5.76 ± 0.22	5.46 ± 0.29	1.83 ± 0.22
(*n* = 30)	15.51–19.10	5.57–7.35	7.13–8.81	6.32–8.52	3.50–4.16	5.30–6.27	5.07–6.45	1.44–2.34
15 km S								
Naranjal	17.84 ± 1.46	6.68 ± 0.48	8.24 ± 0.58	8.06 ± 0.38	4.14 ± 0.25	6.07 ± 0.33	5.54 ± 0.44	1.83 ± 0.12
(*n* = 5)	16.04–19.38	6.25–7.37	7.51–9.10	7.58–8.45	3.75–4.36	5.57–6.39	4.92–6.06	1.64–1.94

ble in ventral view. External nares oval, located dorsolaterally, at about one third the distance between anterior margin of snout and anterior margin of eye.

In profile (Fig. 4) body depressed (BW/BH = 1.4), flattened ventrally, snout rounded. Oral disc posteriorly emarginated. Spiracle sinistral, cylindrical inside the body wall, but protruding laterally, its tip closer to the vent than to the eye, positioned ventrolaterally and oriented posterodorsally. Spiracle opening rounded, situated at level of the hind limbs.

Tail musculature conspicuous, decreasing in size towards the tip of tail. Tail dorsal fin not extending onto body, slightly convex, and reaching its higher size at about midtail. Tail tip nearly rounded. Ventral fin almost straight, beginning just posterior to the vent, about equal size along its length. Anal tube dextral, tubular, linked to caudal muscle. Limb buds of about equal length than diameter. No lateral line or glands.

Oral disc ventral (Fig. 4), transversely elliptical; anterior labium bearing marginal papillae interrupted by a medial, dorsal gap extending two-fourths of the anterior margin of labium; posterior labium emarginated, bordered by two rows of small rounded papillae. LTRF 1/3; A2 = 7 + 3 labial teeth, interrupted by a medial gap; P1 = 14 + 17 labial teeth, interrupted by a right gap; P2 = 5 labial teeth; and P3 = 32 labial teeth. Jaw sheaths thin, serrated at inner side, upper nearly straight, lower angular and shorter than anterior.

In preservative, dorsum, flanks and caudal muscle pigmented by scattered melanophores arranged in a pattern depicted on Fig. 4. Unpigmented areas include a small round spot between eyes at level of anterior margin of eyes, irregular areas around eyes, nares, spiracle, vent tube, fins, distal tail muscles, oral disc, and venter. Tail musculature pigmented with scattered melanophores mostly on the anterior half. Tail fins transparent except on tip of tail, at which melanophores are denser than in adjacent areas forming a discrete, conspicuous mark. Venter of body nearly transparent but with scattered melanophores forming a transverse, diffuse stripe that

TABLE 3.—Measurements (in mm) of developmental stages (sensu Gosner, 1960) of four tadpoles of *Physalaemus guayaco* sp. nov. Abbreviations are: TL = total length; BL = body length; BW = body width; BH = body height; TAL = tail length; ED = eye diameter; ODW = oral disc width; IOD = interorbital distance (measured between centers of pupils); IND = internarial distance (measured between centers of narial apertures); MTH = maximum tail height; TMH = tail muscle height; TMW = tail muscle width.

	Stage			
Variable	A (28)	B (29)	C (29)	D (31)
TL	13.3	16.2	18.0	18.9
BL	4.9	6.0	7.0	7.1
BW	3.7	4.8	4.9	5.0
BH	2.8	3.4	3.7	4.0
TAL	8.3	10.2	11.1	11.9
ED	0.7	0.8	0.9	1.0
ODW	1.4	1.6	1.6	1.8
IOD	1.7	2.1	2.2	2.4
IND	0.9	1.1	1.2	1.1
MTH	2.6	3.2	3.7	3.3
TMH	1.3	1.4	1.7	2.1
TMW	0.9	1.3	1.4	1.4

crosses the body at level of anterior branquial baskets. Color in life unknown.

Tadpole variation: Stage 29 (QCAZ 24006B–C) and Stage 31 (QCAZ 24006D) tadpoles are similar in shape and color pattern to the one described previously. Differences are noted on the LRTF, the amount of labial teeth and its distribution. QCAZ 24006B and 24006D have a LRTF 2/2, whereas QCAZ 24006C has 2/3. In QCAZ 24006B, the number of labial teeth and its distribution is A1 =14, A2 = 17 + 18, P1 = 23, P2 = 28, whereas in QCAZ 24006C is A1 = 3 + 21 + 3, A2 = 16 + 20, P1 = 21 + 24, P2 = 39, P3 = 5, and in QCAZ 24006D is A1 = 49; A2 = 12 + 14; P1 = 44, P2 = 36. A2 and P1 bear larger teeth than the outer rows. A brown tail tip mark is present in all of them and is more conspicuous in C.

Comparisons: Among the species in the *P. pustulosus* group, only the larvae of *P. coloradorum* (Cannatella and Duellman, 1984), *P. petersi* (Duellman, 1978) and *P. pustulosus* (Breder, 1946; Kenny, 1969; Starret, 1960) have been described. They differ from *P. guayaco* by lacking a discrete brown mark at tip of tail. *Physalaemus guayaco* differs further from *P. petersi* by lacking paired elliptical paravertebral glands and by having a different dorsum color pattern (Fig. 4).

Distribution and ecology.—*Physalaemus guayaco* has been recorded from four localities in western Ecuador (Provincia del Guayas) between 32–92 m of altitude. Maximum straight-line distance between localities is 89 km (Fig. 7). The lowlands of western Ecuador are characterized by a low–high precipitation gradient from south to north and west to east. Precipitation is markedly seasonal south of 1° S latitude (rainy season in February–April; Lynch and Duellman, 1997). Among the known localities, annual precipitation ranges from 420–1348 mm (15 km S Naranjal, and 11 km N Cerro Masvale, respectively) with only 27–52 mm falling during the three driest months of the year (July–September at Cerro Masvale); average annual temperature ranges between 24.6 and 25.2 C (Cerro Masvale and 15 km S Naranjal). Cerro Masvale is a private, protected reserve adjacent to a national protected area, Reserva Ecológica Manglares-Churute.

Localities are in Lowland Deciduous Costa Forest (20 km E Durán, 11 km N Cerro Masvale, 15 km S Naranjal), and Lowland Semideciduous Costa Forest (Cerro Masvale; vegetation types are as defined by Sierra et al., 1999 and Cerón et al., 1999). These vegetation types are located between 50 m and 400 m of altitude, covering 25,673 km^2 (10.3% of Ecuador's area). The forest is dryer and the terrain has lower tree densities than evergreen forests. The trees are generally below 20 m and the under-story can be dense with abundant herbaceous plants. Some tree species loss their leaves during the dry season (Cerón et al., 1999). Due to the dry conditions, amphibian diversity is low. Human impact on the natural cover has been severe in this region. More than half of the land cover is being used for agriculture and cattle rising (estimate from AEE, 2000).

All individuals were found in open areas where the original natural vegetation has been partly or completely removed by humans. At Cerro Masvale, in 20 February 2002, *P. guayaco* and *P. randi* were calling from ditches and small ponds in pastures near the buildings of Fundación Andrade field station. On 23 March 2003, only *P. randi* were calling there. However, *P. guayaco* were calling at a nearby flooded rice field (not surveyed in 2002). *Physalaemus randi* were not found at the rice field. At 11 km N Cerro Masvale, a large chorus of *P. guayaco* was calling from a flooded rice field (31 males and 1 female were collected). At the same site, a few *P. randi* were calling (two males collected). At 15 km S Naranjal, males were calling from ponds, less than 20 m away from the highway. *Scinax quinquefasciatus* was calling syntopically.

Our observations of reproductive activity took place in February 2002 and March 2003, during the rainy season. Males begin calling shortly after dusk. Males call from small ponds (down to 10 cm diameter) and ditches while floating in a few centimeters of water, usually concealed by herbaceous vegetation. Amplexus and egg deposition take place at the same sites where choruses call. *Physalaemus guayaco* constructs floating foam nests during amplexus. While the female discharges the egg masses, the male beats them with his legs to produce the foam. On 20 March 2003, a submerged pair of amplectant frogs (QCAZ 23443–44) was captured, among vegetation. Two hours after capture they were placed on a plastic container with water, they begun to build a foam nest. The male kicked the eggs with rapid movements in outbursts that lasted 2–3 seconds with intervals of approximately 10 seconds (time was measured from digital video). During the intervals, the male rested with his legs semi-extended in the foam (angle between the thigh and shank ~70°).

Call.—Acoustic parameters of the advertisement call of five *P. guayaco* are shown in Table 1. The call consists of two components: (1) a single, short note consisting of 3–5 amplitude modulated pulses, followed by (2) a whine-like note (its first harmonic is a nearly pure tone with a descending frequency sweep; Fig. 3, Table 1). Although both components have harmonic structure, the harmonics are clearly defined only in the second component (up to six). One male (QCAZ 19562, call QCAZ [S] 19562; SVL = 19.15 mm) in a chorus at Cerro Masvale, was recorded at 2300 h on 19 February 2002 while calling partly submerged in 2 cm of water, 2 m from a ditch, on a grassy area (water temperature 26.6 C; air temperature 24.8 C). The average duration of the first component is 35.2% of the call (range 33.7–36.0; *n* = 10). In the first component the dominant frequency is always in the second harmonic (mean dominant frequency = 3.200 kHz; *n* = 10), whereas in the second component, the dominant frequency is always in the first harmonic (mean dominant frequency = 1.080 kHz; *n* = 10). The fundamental frequency of the first component is approximately 0.5 kHz higher than that of the second component. Measurements for calls of QCAZ 23510 (air = 28.0 C; water = 27.8 C; SVL = 16.13 mm), QCAZ 23512 (air = 27.7 C; water = 27.7 C; SVL = 16.02 mm), and QCAZ 23652 (air = 28.5 C; water = 26.6 C; SVL = 19.09 mm) are also shown in Table 1. As described in QCAZ 19562, in these calls the dominant frequency of the first component is always above 3 kHz (second harmonic). QCAZ 19561 (call QCAZ [S] 19561; SVL = 17.94 mm) was recorded at 2240 h on at Cerro Masvale on 19 February 2002 (air = 25.8 C; water = 25.8 C). It differs from the other calls because the dominant frequency of the first component is in the first harmonic instead of the second (Table 1). The average duration of the first component is 29.9% of the call (range 25.4–35.8; *n* = 18).

TABLE 4.—Character loading and percentage of explained variance for Principal Components (PC) I–III for seven morphometric variables. To remove the effect of "size", linear regressions were performed between all variables and snout–vent length (SVL). The PC analysis was applied to the residuals from the regressions. Bold figures indicate highest loadings.

Variable	Size-free morphology		
	PC I	PC II	PC III
Residual dorsum width	0.347	**0.737**	−0.329
Residual tibia length	**0.685**	−0.522	0.014
Residual femur length	0.622	−0.115	−0.069
Residual arm length	**0.685**	−0.349	−0.183
Residual head length	0.540	0.046	0.449
Residual head width	0.473	0.701	−0.196
Residual eye-nostril distance	0.153	0.364	**0.810**
Eigenvalue	1.984	1.576	1.043
%	28.3	22.5	14.9

Antiphonal calling is frequent in male choruses. The spectrogram of such behavior is shown in Fig. 3 (between QCAZ 23508 and a noncaptured individual). The frogs were recorded on 23 March 2003 at 2053 h in a flooded rice field in Cerro Masvale (air = 27.2 C; water = 27.8 C). *Physalaemus guayaco* has a call very similar to that of *P. montubio* (Ron et al., 2004; Fig. 3). However, the sound frequency is slightly lower in *P. montubio* (Diagnosis).

Morphometric comparisons.—Three components with eigenvalues >1.0 were extracted from the PCA of 215 specimens belonging to four species of the *P. pustulosus* group, including *P. guayaco*. The axes accounted for 65.7% of the total variation. Along PC I, the highest loadings were for arm and tibia length. Along PC II, the highest were dorsum and head width (Table 4). There is a wide overlap between the morphometric space of *P. guayaco* and *P. randi* (Fig. 10). The overlap is less extensive between *P. guayaco* and *P. montubio*, especially along PC I (Fig. 10).

In the DFA classification procedure, 37 out of 55 specimens of *P. guayaco* were classified correctly. The misclassified specimens were assigned to *P. pustulatus* (nine specimens), *P. montubio* (five), and *P. randi* (four). The high rate of correct group assignment indicates some degree of morphometric distinctiveness among species, independent of overall size differences. The posterior probability for the classification of each specimen as *P. guayaco* is shown in Fig. 11. Mean probabilities are: *P. guayaco* = 0.48 (SD = 0.20, *n* = 55); *P.*

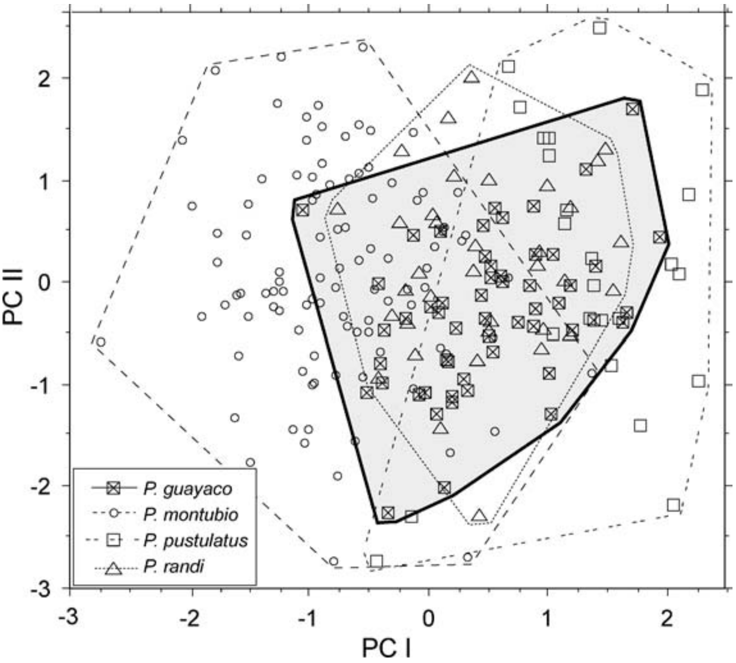


FIG. 10.—Axes I and II from Principal Components Analysis based on seven size-corrected morphological variables for *Physalaemus guayaco* (55 specimens), *P. montubio* (101), *P. pustulatus* (24), and *P. randi* (35).

pustulatus = 0.22 (SD = 0.23; n = 24); *P. randi* = 0.18 (SD = 0.18; n = 35); and *P. montubio* = 0.15 (SD = 0.18; n = 101). According to the DFA of size-corrected morphology, the species most similar to *P. guayaco* is *P. pustulatus*; the least is *P. montubio*.

Phylogenetic Relationships

An expansion of the phylogenetic analysis based on morphological characters presented by Cannatella et al. (1998) shows that *P. guayaco* is part of a clade that includes the species of the *P. pustulosus* group that occur west of the Andes in northwestern South America (Fig. 1); this clade is sister to a clade distributed in Central America and east of the Andes (*P. petersi* + *P. pustulosus*). The synapomorphies that support the western South American clade are (1) absence of a tarsal tubercle, and (2) narrow stalk of the alary process of the hyoid. The topology within this clade is not well supported and should be interpreted with caution because the only informative character is SVL.

The phylogenetic analysis of mtDNA (2409 bases, 348 parsimony-informative) yields a single most-parsimonious tree (Fig. 1). All clades have a strong support except for a bootstrap of 67% for clade (*P. guayaco* + *P. coloradorum*). The maximum likelihood analysis (best-fit model GTR + Γ) yielded a single tree with a branching pattern identical to that from parsimony. The sister-species relationship between *P. montubio* and *P. randi* verifies the distinctiveness of *P. montubio* and *P. guayaco*, despite the similarity in external morphology and advertisement call. Low intraspecific genetic differentiation is evident in all species (uncorrected p-distances range 0.0004–0.0025) despite geographic distances as high as 220 km (between both populations of *P. montubio*). Genetic distances between four *P. guayaco* populations range between 0.0004 (QCAZ 23656 from 15 km S Naranjal vs. QCAZ 19561 from Cerro Masvale) and 0.0025 (QCAZ 23652 from 15 km S Naranjal vs. QCAZ 23533 from 11 km N Cerro Masvale); genetic distances between species range between 0.0972 (*P. pustulatus* QCAZ 23320 vs. *P. coloradorum* QCAZ 19418) and 0.0284 (*P. montubio* QCAZ 23190 vs. *P. randi* QCAZ 19559).

The status of the populations of *Physalaemus*, from southwestern Ecuador (Pasaje, Provincia del Oro), which have advertisement call and external morphology similar to that of *P. randi* is still unresolved. We will refer to them as *P. randi* although it is still unclear if they deserve to be considered a separate species.

DISCUSSION

Complex Calls are Common Among Species of the P. pustulosus *Group*

The clade including the four smallest species (mean male SVL < 21 mm; Fig. 1A) is characterized by calls with shorter duration. The calls of *P. guayaco* and the recently described *P. montubio* and *P. randi* (Ron et al., 2004) are unique in having an amplitude-modulated component with several well defined pulses (first component) prior to the frequency sweep (second component) ubiquitous in members of the group (Fig. 3). Previous accounts of call evolution and sexual selection in the *P. pustulosus* group have referred to the first component as a "prefix" (in *P. randi* from southern Ecuador, mistakenly ascribed to *P. pustulatus*) and to the second component as a "whine" (e.g., Ryan, 1997; Ryan and Rand, 1993*a*; Ryan and Rand, 2001).

The spectral features of both components are different in: (1) fundamental frequency, which is 0.2–0.7 kHz higher in the first component than in the second (Table 1; Ron et al., 2004); and (2) sound energy distribution, which in the second component always peaks in the first harmonic (at about 0.9–1.2 kHz) but in the first component tends to be allocated equally between the first and the second harmonics (often more energy is in the second harmonic in *P. guayaco*; peak at 2.7–3.4 kHz; Table 1; Fig. 3).

The distinct spectral features of both components are likely to match the sensory sensitivity of females. Many studies have demonstrated a correlation between auditory tuning and the frequency of the bands with high-energy allocation in conspecific advertisement calls (reviewed by Gerhardt and Schwartz, 2001). The auditory tuning of *Physalaemus* shows two spectral ranges of enhanced sensitivity, one from 0.1 to 1.100 kHz, which presumably represents amphibian papilla tuning, and another from 2.100 to 2.550 kHz (presumably basilar papilla tuning; Wilczynski et al., 2001, based on measurements on *P. petersi*, *P. pustulosus*, *P. coloradorum*, and *P. randi*). Studies of sexual selection in *P. pustulosus* suggest that matching between sound frequency of the advertisement call and female auditory tuning may influence female mate choice (Ryan and Rand, 1999). *Physalaemus pustulosus* has a complex advertisement call with two main components: (1) a whine-like frequency sweep (likely homologous to the second component of *P. guayaco*,

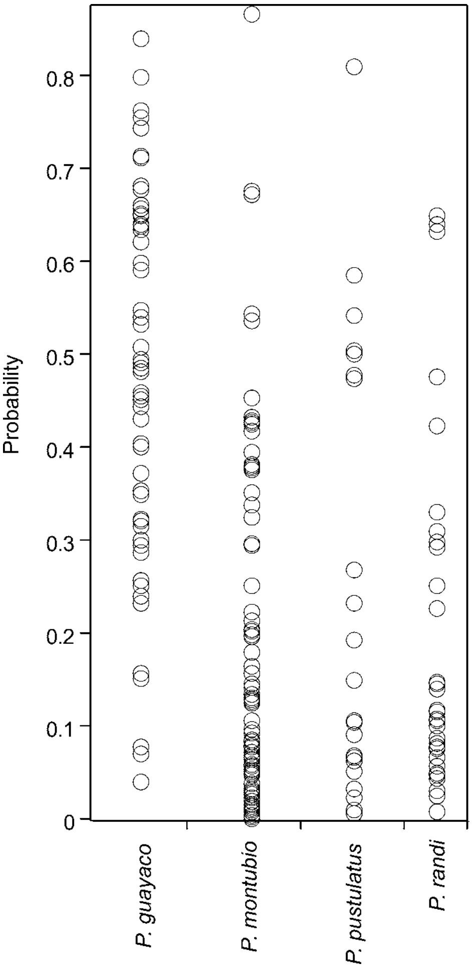


FIG. 11.—Posterior probabilities of the assignment to *Physalaemus guayaco* of each of 101 specimens of *P. montubio*, 24 of *P. pustulatus*, 35 of *P. randi*, and 55 of *P. guayaco* derived from Discriminant Function Analysis of seven size-corrected morphometric variables. *Physalaemus montubio* specimens have the lowest average probabilities of being misclassified as *P. guayaco* (in 70.3% $P < 0.20$).

P. montubio and *P. randi*) that stimulates primarily the amphibian papilla; and (2) a subsequent and facultative higher frequency "chuck" (peak energy at 2.55 kHz) that stimulates the basilar papilla (Ryan et al., 1990). The whine is necessary and sufficient to attract females. However, females prefer calls that have a whine and one (or more) chucks to whine-only calls (Rand and Ryan, 1981). It has been suggested that females prefer the whine-chuck calls because they are a better match for the spectral sensitivity of the amphibian and basilar papillae (Wilczynski et al., 2001).

We propose that the first component of the calls of *P. guayaco*, *P. montubio*, and *P. randi* have spectral features that stimulate the basilar papilla in a similar manner to the chuck of *P. pustulosus*. We base this prediction on the following evidence: (1) in several species of anurans (including *P. pustulosus*) there is a match between auditory tuning and the frequencies emphasized in the conspecific advertisement call (Gerhardt and Schwartz, 2001); (2) the frequency of the second harmonic of the first component is close to the enhanced sensitivity range of the basilar papilla (2.549 kHz in *P. randi* from Pasaje, Ecuador; Wilczynski et al., 2001 [referred as "*P. pustulatus*" in that publication]); (3) the peak frequency of the second harmonic of the first component is similar to that of the "chuck"; and (4) females of *P. pustulosus* prefer their conspecific whines with an artificially added first component (from calls of *P. randi* from Pasaje) over whine-only calls, suggesting that the first component may play a functional role similar to that of the "chuck" (Ryan and Rand, 1993a). In other words, the first component of the calls of *P. guayaco*, *P. montubio*, and *P. randi* may be functionally similar to the "chuck" of the complex calls of *P. pustulosus* because it has a significant amount of energy that should match the spectral sensitivity of the basilar papilla. As in *P. pustulosus*, the second component has most of its energy matching the spectral sensitivity of the amphibian papilla. If in fact females prefer calls that match the frequency sensitivity of both papillae, we might expect that female *P. guayaco*, *P. montubio*, and *P. randi* will show a preference for complex calls (chuck + second component or first component + second component) over simple calls (second component only).

Physalaemus coloradorum also belongs to the clade of small species. The following discussion is based on averages from advertisement calls of five male *P. coloradorum* from 5 km NW La Florida, Provincia del Pichincha (QCAZ 19412, 19416–8, and 19441). The call of *P. coloradorum* has two components that resemble those of the calls of *P. guayaco*, *P. montubio*, and *P. randi* (Fig. 3). The first component in *P. coloradorum* contains about 65% of the energy of the call and also is amplitude-modulated. In contrast with *P. guayaco*, *P. montubio*, and *P. randi*, the pulses of the first component are weakly defined in *P. coloradorum* (Fig. 3). As in all other members of the species group, the second component is a downward frequency sweep. The dominant frequency of the first component is approximately 0.149 kHz higher than that of the second. Although the dominant frequency of the first component is in the first harmonic, at 1.104 kHz, 45% of its sound energy is allocated above 1.2 kHz and therefore should stimulate the basilar papilla. In fact, the frequency with the most energy in the second harmonic of the first component (2.227 kHz) matches the best excitatory frequency of the basilar papilla (2.228 kHz; Wilczynski et al., 2001). It is unknown whether this level of stimulation of the basilar papilla can increase the attractiveness of the calls to females although this scenario is plausible considering that the energy >1.2 kHz in the first component is 26% of the total energy of the call of *P. coloradorum* compared to 7% for the chuck of *P. pustulosus* (Ryan and Rand, 1990). Female *P. coloradorum* prefer conspecific calls to which chucks of *P. pustulosus* have been artificially appended versus normal conspecific calls (Ryan and Rand, 1993*b*).

It has been assumed that the advertisement calls of the South American *Physalaemus* distributed west of the Andes are characterized by being simple, with a dominant frequency <1.0 kHz, and very little energy in the range of sensitivity of the basilar papilla (e.g., Ryan, 1990; Ryan and Rand, 1993*a*; Wilczynski et al., 2001). However, the calls of the recently described species (herein and in Ron et al., 2004) and even that of *P. coloradorum* have a complex structure consisting of two components with different spectral features and a significant amount of energy in the range of frequency sensitivity of both the amphibian and basilar papilla. These new findings make necessary a reevaluation of previous species-level analyses of call evolution and female choice that have assumed that calls with high frequency components are restricted to the clade (*P. pustulosus* + *P. petersi*). Unfortunately, that endeavor is beyond the scope of the present article.

Resumen

Describimos una nueva especie del género leptodactílido *Physalaemus* de las tierras bajas del occidente del Ecuador. La nueva especie pertenece al grupo de especies *P. pustulosus*. Se diferencia de otras especies del grupo por su tamaño más pequeño, textura de la piel, características del renacuajo, y llamado de anuncio. La nueva especie presenta una amplia variación en tamaño y patrón de coloración a nivel intra- e inter-poblacional. Esta variación es similar a la observada en *P. montubio* y *P. randi* haciendo difícil su diagnóstico en base a morfología. Un análisis filogenético basado en caracteres morfológicos evidencia que la nueva especie pertenece a un clado distribuido al oeste de los Andes en Ecuador y el Norte del Perú, hermano de (*P. petersi* + *P. pustulosus*). Una filogenia basada en ADN mitocondrial muestra que *P. montubio* y *P. randi* forman un clado hermano de (*P. coloradorum* + *P. guayaco* sp. nov.). Los cantos de estas tres especies tienen dos componentes con características acústicas diferentes entre sí, cuyas frecuencias sonoras posiblemente se correlacionen con la sensibilidad de frecuencias del oído de hembras conespecíficas, de manera similar a lo observado en los cantos complejos de *P. pustulosus*. La nueva especie se encuentra presente en hábitats predominantemente secos, el Bosque Deciduo de Tierras Bajas de la Costa y el Bosque Semideciduo de Tierras Bajas de la Costa.

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Appendix I

Examined Specimens

Cleared-and-stained specimens are designated with C & S.

Physalaemus coloradorum: Ecuador: Provincia del Pichincha: Tinalandia (AMNH 111556); 1–5 km NW from La Florida, 1003 m (QCAZ 2975, 19373–74, 19417–18, 19439–41); Near Alluriquín (QCAZ 19336); on road between Alluriquín and Santo Domingo de los Colorados (QCAZ 19294); Santo Domingo de los Colorados (KU 187271); 6 km E Santo Domingo de los Colorados (USNM 212256); Río Baba, 5–10 km SSW Santo Domingo de los Colorados (KU 146194); Río Cupa (USNM 196869).

Physalaemus montubio: Ecuador: Provincia de Manabí: Pedernales, 85 m (QCAZ 23197–3201); Estero Ancho, 329 m (QCAZ 23188–192); Río Chico, 24 m (QCAZ 23252–59); road between Canoas and San Vicente, 10–20 m (QCAZ 23204–05, 23207–09, 23231–36); San Vicente, 10 m (QCAZ 23210, 23212, 23214–18, 23220–24, 23226–30); Portoviejo, 56 m (QCAZ 23237–247, 23249); Puerto Rico, 30 m (QCAZ 19366, 19375, 19377–380, 19511, 19515–17, 19519–522, 19524 [holotype], 19526–527, 19530–33, 19549–550, 19552, 19555–57). Provincia del Guayas: Montañita, 20 m (QCAZ 23271–282); on road between El Palmar and Balsas, 5–110 m (QCAZ 23323, 23325, 23330–31, 23373–77, 23379–81, 23386–392, 23397).

Physalaemus petersi: Bolivia: Cochabamba: 6.5 km N Chipiriri, 260 m (KU 135513–16). Ecuador: Provincia de Orellana: Estación Científica de la Universidad Católica del Ecuador, Parque Nacional Yasuní, 240 m (QCAZ 14733–38, 18282 [tadpole]).

Physalaemus pustulatus: Ecuador: Provincia de Manabí: Puerto Rico, 10 m (QCAZ 19355, 19513–14, 19518, 19523, 19537 [C & S]), 19541–42, 19545–48, 19551, 19553–54. Provincia de Los Ríos: Patricia Pilar, 200 m (QCAZ 19538–40, 19605–14, 19745–47, 19748 [C & S]). Provincia del Guayas: Guayaquil (MCZ 7666 [holotype]); Isla Puná (CAS 5408); Cerro Blanco (QCAZ 23427).

Physalaemus randi: Ecuador: Provincia del Guayas: 11 km N Cerro Masvale, 40 m (QCAZ 23461, 23523); Cerro Masvale, 92 m (19558–560, 19563 [holotype], 19564–66, 19569, 19571, 19573–75, 19576 [C & S], 19577–78, 19579–582 [all C & S],

Appendix II

Data matrix for morphology-based phylogenetic analysis.

	1	2	3	4	5	6	7	8	9	10	11	12	13
P. petersi	1	0	1	1	1	1	1	1	1	0	0	1	1
P. pustulatus	0	1	1	1	1	0	0	1	0	1	1	0	2
P. pustulosus	1	0	1	1	1	1	0	1	1	0	0	1	3
P. randi	0	1	1	1	1	0	0	1	0	1	1	0	4
P. montubio	0	1	1	1	1	0	0	1	0	1	?	0	5
P. guayaco	0	1	1	1	1	0	0	1	0	1	?	0	6
P. coloradorum	0	1	1	1	1	0	0	1	0	1	1	0	7
P. enesefae	0	0	0	0	0	0	0	0	0	0	0	0	8
P. ephippifer	0	0	0	0	0	0	0	0	0	0	0	0	9

Character 13 (stepmatrix)

	0	1	2	3	4	5	6	7	8	9
0	—	998	740	784	19	272	0	258	606	918
1	998	—	258	214	979	726	998	740	392	80
2	740	258	—	44	721	468	740	482	134	178
3	784	214	44	—	765	512	784	526	178	134
4	19	979	721	765	—	254	19	239	588	899
5	272	726	468	512	254	—	272	15	334	646
6	0	998	740	784	19	272	—	258	606	918
7	258	740	482	526	239	15	258	—	349	660
8	606	392	134	178	588	334	606	349	—	312
9	918	80	178	134	899	646	918	660	312	—

Appendix III

Locality data and Genbank accession numbers for *Physalaemus* included in the phylogeny based on DNA mitochondrial sequences. All specimens were collected in Ecuador and are deposited at the collection of the Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ).

Species	QCAZ museum no.	GenBank accession no.	Locality
P. coloradorum (1)	19417	AY834181	Pichincha: 5 km NW La Florida
P. coloradorum (2)	19418	AY834182	Pichincha: 5 km NW La Florida
P. guayaco	19561	AY834172	Guayas: Cerro Masvale
P. guayaco	23445	AY834173	Guayas: 11 km N Cerro Masvale
P. guayaco	23533	AY834175	Guayas: 11 km N Cerro Masvale
P. guayaco	23652	AY834176	Guayas: 15 km S Naranjal
P. guayaco	23656	AY834174	Guayas: 15 km S Naranjal
P. montubio	23190	AY834177	Manabí Estero Ancho, 52 km W El Carmen
P. montubio	23271	AY834178	Guayas: Montañita
P. pustulatus	19551	AY834183	Manabí: Puerto Rico
P. pustulatus	23320	AY834184	Guayas: near Balsas
P. randi	19559	AY834179	Guayas: Cerro Masvale
P. randi	23425	AY834180	Guayas: Cerro Blanco