Taxonomic reassessment of *Mannophryne trinitatis* (Anura: Dendrobatidae) with a description of a new species from Venezuela

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A new species of dendrobatid frog of the genus *Mannophryne* is described from Península de Paria, Estado Sucre, Venezuela. The new taxon, closely related to *M. trinitatis*, an endemic species of Trinidad, was for a long time considered a continental population of this species. External morphology and call characteristics provide taxonomic distinction between the continental and the insular species, while mitochondrial DNA supports a sister taxon relationship between them. The new species differs from the other members of the genus by its small size (mean snout–vent length 21.5 mm in females, 19.4 mm in males), unpigmented and not well-defined collar and reduced foot webbing. Additionally, the new taxon can be distinguished from *Mannophryne trinitatis* by: 1) dorsal coloration with a well contrasted pattern; 2) pale dorsolateral stripes not well defined; 3) a paler brown pigmentation on palms and soles; 4) diffuse inguinal stripes; 5) dark markings absent along the anterior margins of forelimbs; 6) advertisement call with a single frequency-modulated note; and 7) differences in 16S and COI mtDNA sequences. Comments on natural history, biogeography and conservation status are provided.

Key words: Amphibia, *Mannophryne*, taxonomy, mtDNA, biogeography, Sucre State, advertisement call

INTRODUCTION

Species of the genus *Mannophryne* (La Marca, 1992) are characterized by the presence of a dark collar, yellow throat (mostly in adult females) and elaborate behaviour (throat displays and toe tip jumping). Twelve species of *Mannophryne* are currently recognized (Frost, 2004): *M. caquetio* Mijares-Urrutia & Arends, 1999, *M. collaris* Boulenger, 1912, *M. cordilleriana* La Marca, 1994, *M. herminae* Boettger, 1893, *M. lamarcai* Mijares-Urrutia & Arends, 1999, *M. larandina* Yústiz, 1991, *M. neblina* Test, 1956, *M. oblitterata* Rivero, 1984, *M. olmonae* Hardy, 1983, *M. riveroi* Donoso-Barros, 1965, *M. trinitatis* Garman, 1887 and *M. yustizi* La Marca, 1989. The monophyly of the group is supported by mtDNA analyses (Manzanilla et al., 2003; Vences et al., 2003a).

The geographic distribution of *Mannophryne* ranges along the western and northern mountainous regions of Venezuela, from the Andean Cordillera of Mérida to the Península de Paria and Trinidad and Tobago. Ten species of *Mannophryne* are endemic to Venezuela; *M. trinitatis* is thought to occur in Venezuela and Trinidad (see further discussion), while *M. olmonae* is endemic to Tobago.

The taxonomic status of continental populations currently included within *M. trinitatis* is at least doubtful. *Mannophryne trinitatis* was originally described by Garman (1887) as *Phyllobates trinitatis.* The type locality of the syntypes provided in the original description, "Trinidad", does not allow the precise allocation of the type series to a particular locality. The species *M.*
herminae was originally described from Puerto Cabello, central Venezuelan Coastal Range, under the name *Prostherapis herminae*, but it was later considered by Barbour & Noble (1920) as a junior synonym of *M. trinitatis*. By doing so, these authors implicitly extended the range of *M. trinitatis* to central Venezuela. Several authors (Lutz, 1927; Hellmich, 1940; Mertens, 1957; Ginés, 1959; Sexton, 1960; Test, 1963; Dixon & Rivero-Blanco, 1985) have followed Barbour & Noble's criteria, treating *M. herminae* as a synonym of *M. trinitatis*. In a subsequent revision of the genus, La Marca (1992, 1994) considered *M. herminae* as a full species and provided morphological evidence supporting the distinctiveness of both taxa. La Marca (1994) assigned specimens of *Mannophryne* from the southern versant of the central Venezuelan Coastal Range and eastern Coastal Range (from Estado Guárico to Península de Paria, Venezuela) to *M. trinitatis*, but stated that the taxonomic status of these populations deserved further investigation "since they likely represent different taxa". Finally, Kaiser et al. (2003) considered that "the name applied to *Mannophryne* populations occurring in the Northern Range of Trinidad should remain as *M. trinitatis*, as that is the type locality for the species" and suggested that the populations from Trinidad's Central Range and the Venezuelan coastal Cordillera should be referred to as *Mannophryne* cf. *trinitatis*.

A detailed morphological comparison between specimens currently assigned to *M. trinitatis* from Trinidad and from Península de Paria in northeastern Venezuela

Table 1. List of specimens examined, type locality, museum numbers and GenBank accession numbers.

Species	Collection locality	Museum no./reference	Accession no.
Mannophryne venezuelensis	Venezuela. Type locality. San Juan de Las Galdonas–San Juan de Unare Road	ULABG-6706	DQ084067
Mannophryne venezuelensis	Venezuela. Type locality. San Juan de Las Galdonas–San Juan de Unare Road	ULABG-6707	DQ084068
Mannophryne venezuelensis	Venezuela. Type locality. San Juan de Las Galdonas–San Juan de Unare Road	ULABG-6708	DQ084069
Mannophryne venezuelensis	Venezuela. San Juan de Unare, Estado Sucre	ULABG-6709	DQ084070
Mannophryne trinitatis	Trinidad. Mt. Saint Benedict	GLAHM:128892	AY255841
Mannophryne trinitatis	Trinidad. Maracas Bay	GLAHM:128869	DQ084071
Mannophryne trinitatis	Trinidad. Maracas water fall	GLAHM:128924	AY 255840
Mannophryne trinitatis	Trinidad. Matelot	GLAHM:128974	AY191229
Mannophryne trinitatis	Trinidad. Tamana cave	GLAHM:128949	AY191228
Mannophryne collaris	Venezuela. La Entrada. Vicinity of Mérida	La Marca et al., 2002	AJ430675
Mannophryne herminae	Venezuela. La Entrada. Aragua	Vences et al., 2003a	AY263219
Nephelobates molinarii	Venezuela. Near Bailadores	Vences et al., 2003a	AY263216
Colostethus humilis	Venezuela. Trujillo	La Marca et al., 2002	AJ430673

reveals consistent differences between them. This is further supported by differences in call characteristics and mitochondrial DNA (mtDNA). We thus consider that the populations of *Mannophryne* from Península de Paria are taxonomically differentiated from the populations in Trinidad, and should be recognized as a new taxon. The main goal of this paper is to describe this new species of *Mannophryne* from Venezuela.

MATERIALS AND METHODS

Terminology and methods for descriptions were adapted from Caldwell & Lima (2003) and Mijares-Urrutia & Arends (1999a,b). The scheme of webbing formulae is that employed for most of the species descriptions within the genus (La Marca, 1994, 1997). Measurements of preserved adult specimens (in mm) were made with a digital calliper (Mitutoyo® calliper) with a 0.01 mm precision under a 10x lens. The following measurements were analysed: snout–vent length (SVL); head length, from posterior corner of mouth to tip of snout (HL); head width, at level of angle of jaws (HW); snout length, from anterior corner of eye to snout tip (SL); eye-to-naris distance, from anterior corner of eye to centre of naris (EN); internarial distance, between inner border of naris (IN); horizontal length of eye, from anterior to posterior corner of eye (EYE); interorbital distance, between inner border of eyelids (IO); horizontal length of tympanum (TYM); forearm length, from proximal edge of palmar tubercle to outer edge of flexed elbow (FAL); hand length, from proximal edge of palmar tubercle to tip of finger III (HAND); tibia length, from outer edge of flexed knee to heel (TL);

foot length, from proximal edge of outer metatarsal tubercle to tip of toe IV (FL). Sex of specimens was assessed by gonad inspection. Geographical coordinates were obtained with a GPS Garmin® 12. Abbreviations for Venezuelan museum names follow Bisbal & Sánchez (1997). Abbreviations for non-Venezuelan museums are: GLAHM: The Hunterian Museum (Graham Kerr Building), University of Glasgow; KU: University of Kansas, Museum of Natural History.

Specimens collected during this work were fixed and preserved under standard museum protocols. Staging of tadpoles followed Gosner (1960). Description of the larvae is based on 23 larvae at stage 24, collected at San Juan de Las Galdonas, Estado Sucre, Venezuela (KU 181981).

Tissues from five specimens of *M. trinitatis* from five localities in Trinidad, and four specimens of *Mannophryne* sp. nov. from two localities in Estado Sucre, Venezuela (including the type locality), were preserved in 95% ethanol for molecular studies. Total genomic DNA was extracted from preserved tissues by digestion with proteinase K, followed by phenol-chloroform-isoamyl alcohol standard treatment (Hedges et al., 1991). A region of approximately 489 bp of the ribosomal 16S mtDNA gene was sequenced using the primers "16Sar" and "16Sbr" (Palumbi, 1996). Double-stranded fragments were amplified using the polymerase chain reaction (PCR) in a Gene Amp® thermocycler (PCR System 9700, Applied Biosystems). Reactions were performed in a total volume of 26 µl: 2.5 µl buffer 10X (Biotools standard buffer); 1µl of each primer 0.1 µM, 0.3 µl of Taq polymerase (Biotools DNA Polymerase) 5U/µl, 2.5 µl of dNTP, and 0.2 µl of $MgCl_2$ 0, 5µM. Additional sequences

of approximately 751 bp of the cytochrome oxidase I (COI) mtDNA were sequenced (seven specimens from five localities) using the primers COIF and COIR (San Mauro et al., 2004), using the same procedure.

Double strand templates from PCR were cleaned using sodium acetate and ethanol. We used double strand DNA as the template for cycle sequencing reactions in 10 µl total volume using standard conditions. The amplified fragments were sequenced by JM at the Museo Nacional de Ciencias Naturales de Madrid, and MJJ at the University of Glasgow (see Table 1 for GenBank accession numbers for the 16S sequences).

Phylogenetic analyses of the 16S data set were conducted using maximum parsimony (MP) and maximum likelihood (ML) procedures in PAUP* 4.0b10 (Swofford, 2002). All characters were equally weighted and gaps were treated as missing data. For these analyses we use additional sequences already published for some species of Dendrobatidae (Table 1). One species of *Colostethus* of the *C. brunneus* group was used as outgroup. One species of *Nephelobates*, the hypothesized sister group of *Mannophryne* (Manzanilla et al., 2003; Vences et al., 2003a), was also included in the analyses. In order to find the best model of evolution for the data, we used Modeltest 3.06 (Posada & Crandall, 1998) under Akaike's information criterion (Akaike, 1974) to perform the subsequent ML analysis (Felsenstein, 1981). We used nonparametric bootstrapping (1000 pseudoreplicates) to asses the stability of internal branches (Felsenstein & Kishino, 1993).

MP phylogenies (Swofford et al., 1996) were estimated using the heuristic search algorithm. We used 100 randomized input order replicates for all MP analyses to minimize the effect of entry order on the topology of the resulting cladograms. MP analyses were conducted without steepest descent option, and with accelerated character transformation (ACCTRAN) optimization, tree bisection–reconnection (TBR) branch swapping, and zero-length branches collapsed to yield polytomies. We used 1000 nonparametric bootstrap pseudoreplicates to asses the stability of internal branches in the resulting topologies (Felsenstein, 1981; Felsenstein & Kishino, 1993).

Dates of divergence were estimated for 16S rDNA corrected distances on the same basis adopted for Martínez-Solano et al. (2004) for other anurans, corresponding to 0.39–0.40 % divergence per Ma. In the absence of comparative divergence data, estimates for COI were based on Bermingham et al. (1997) for fishes, and also on Knowlton & Weigt (1998) for marine invertebrates, corresponding to 1.2–1.5% per Ma.

We studied the advertisement calls of two males of the new species described herein, along with several male choruses recorded at the type locality on 17 July 2003 between 1500 and 1700 m. Advertisement calls from five males and choruses from an *M. trinitatis* population were recorded at Mount Saint Benedict Trinidad (10°41'N, 61°23'W) during the first week of July 2003. The total duration of analysed calls for each species was approximately 12 minutes. Calls were recorded with a di-

rectional TECT model UEM-81 super-cardioid/cardioid condenser microphone and a Sharp® MD-MT280E(S) portable minidisc recorder. Calls were edited and analysed using Praat® v3.4 (Boersma & Weenink, 1996).

All calls were filtered (band smoothed at 100 Hz) and enhanced at 5 dB (individual calls) or at 20 dB (choruses) to reduce or erase background noise. The following calling parameters were analysed: 1) dominant minimum and maximum frequency at start of note and end of note; 2) dominant note frequency increases between minimum and maximum; 3) note duration; 4) internote duration; 5) duration of note maximum intensity, (6) number of intensity maximum rises per note; 7) paired notes combined duration, from beginning of first note to end of second note; 8) duration between paired notes, from end of first note to beginning of second note; 9) bout duration; 10) number of notes per bout; and 11) interbout duration.

RESULTS AND DISCUSSION

Species description: *Mannophryne venezuelensis* sp. nov. (Figs 1 and 2)

Holotype. EBRG 4924, an adult female collected by L. De Sousa and J. Manzanilla on 7 May 2002, from approximately 4.0 km east of San Juan de Las Galdonas, Municipio Arismendi, Estado Sucre, Venezuela, 10º43'N, 62º48'W, altitude 180 m.

Paratypes. Adult females EBRG 4919, 4922, 4923, and adult males EBRG 4920–4921, (topotypes, same data as holotype). Adult females EBRG 284, 288, 289, 292, and adult male EBRG 290 from San Juan de Las Galdonas, 180 m of altitude, Municipio Arismendi, Estado Sucre, Venezuela. Collected by L. De Sousa and J. Manzanilla on 7 May 2002.

Referred specimens. We studied additional material from several localities within the Península de Paria, Estado Sucre, assigned to *M. venezuelensis* and catalogued as EBRG 2574, Península de Paria, Río El Hoyo, altitude 130 m, collected on 21 April 1993, by R. Rivero; EBRG 2589, 2590, Quebrada Don Pedro, Parque Nacional Península de Paria, altitude 50 m, on 29 September 1993, by R. Rivero and R. Suárez; EBRG 2579, Uquire, Parque Nacional Península de Paria, altitude 150 m, on 25 August 1993, by R. Rivero and F. Bisbal; EBRG 2549–2551, Parque Nacional Península de Paria, Hacienda Solís, altitude 150 m, on 19 February 1993, by R. Rivero and A. Bermúdez.

Etymology. The species name "*venezuelensis*" is a noun in the genitive singular and neuter gender, formed as a geonym making reference to the country of provenance of the type, Venezuela.

Diagnosis. A small-sized species of *Mannophryne*, adult females varying from 19.2 to 23.0 mm SVL, adult males from 18.9 to 20.0 mm SVL. Throat and chest yellow in adult females and grey in adult males. *Mannophryne venezuelensis* differs from *M. collaris*, *M. cordilleriana*, *M. oblitterata* and *M. riveroi* in the presence of reduced foot webbing (well developed in those species) and smaller body size; *M. venezuelensis* differs from *M. herminae*, *M. caquetio* and *M. lamarcai* by showing an
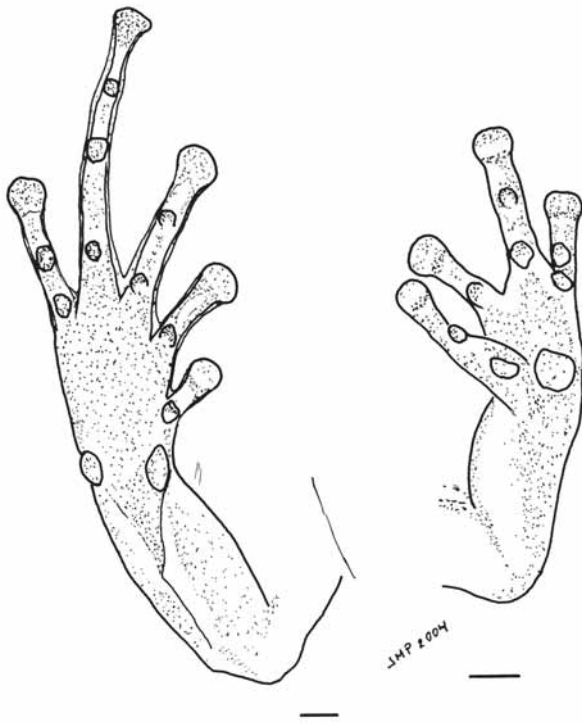
Fig. 1. Ventral view of the right foot and the left hand of *Mannophryne venezuelensis* (Holotype EBRG 4924).

unpigmented and not well defined collar (wide and well defined in these three latter species); also differs from *M. olmonae* in the reduced webbing between toes IV and V (well developed in *M. olmonae*) and the presence of a poorly defined and scarcely pigmented collar. Particularly, *M. venezuelensis* differs from *M. trinitatis* (characters within parenthesis) by the presence of the following combination of characters: 1) poorly defined and scarcely pigmented collar (well defined and solid in *M. trinitatis*); 2) dorsal coloration showing a well contrasted, reticulated pattern (dorsum almost solid brown in *M. trinitatis*); 3) pale dorsolateral stripes not well defined (well defined in *M. trinitatis*); 4) pale brown pigmentation uniform on palms and soles (external margin of palm and soles dark pigmented in *M. trinitatis*); 5) pale inguinal stripe diffused (well defined in *M. trinitatis*); 6) markings almost absent along anterior margins of forelimbs (band-like concentration of melanophores present along anterior part of the arm in *M. trinitatis*); 7) poorly pigmented metatarsal and subarticular tubercles on toes (dark pigmented in *M. trinitatis*); 8) advertisement call characteristics consist of a single frequency-modulated note (two frequency-modulated notes composed of paired pulses in *M. trinitatis*).

Description of the holotype. An adult female (deep convoluted oviducts, mean egg size = 1.1 mm). SVL = 22.1 mm; head just slightly wider than long (head length 7.8 mm, 96% of head width); head length 35% of SVL. Snout broadly rounded in dorsal view, acutely rounded in lateral view. Snout 45% of head length; nares located laterally, opening posterolaterally, slightly protruding, closer to tip of the snout than to eye. Nares partially concealed in their upper edge by a dermal thickening. Canthus rostralis well defined, almost straight. Loreal region almost flat.

Internarial distance 40% of head width. Horizontal eye diameter 2.9 mm. Eye to nostril distance 72.4% of horizontal eye length. Tympanic ring well defined. Horizontal length of tympanum 52% of horizontal eye diameter. Eye–tympanum distance 80% of the horizontal diameter of the tympanum. A rounded, flattened and inconspicuous tubercle at corner of lips. Posterior third of tongue not adherent to mouth floor. Tongue broadly rounded and lightly notched along posterior margin. Small acute teeth present on the maxillae and premaxillae. Vomerine teeth absent. Choanae rounded, medium sized, almost concealed by the border of the palatal shelf of the maxillary arch. Distance between choanae equal to internarial distance. Upper eyelid without tubercles. Skin on top of head smooth; dorsum smooth with scattered inconspicuous tubercles, more distinct towards the posterior end of the back; skin of dorsal surfaces of thighs and shanks shagreened; skin of belly and ventral surface of limbs smooth. Cloacal opening at upper level of thighs. Short cloacal fold completely covering the cloacal vent. Border of cloacal fold entire above vent. Forelimb slender with smooth skin. Hand length 25% of SVL; FAL 20% of SVL. Relative length formula of appressed fingers: III>IV≥II≥I. Palmar tubercle nearly round. Thenar tubercle oval, about twice as long as wide, approximately one-third of the length of the palmar tubercle. Supernumerary tubercles absent. Subarticular tubercles flat top in profile, elevated, rounded to oval in shape. Discs of the fingers slightly wider than long. Larger disc on finger III, covering about one-third of the tympanum when adpressed against it. Webbing between fingers absent. Fingers with lateral fringes. Disc with distinct dorsal scutes. Keels present along the border of second and third finger. Tibia length 47% of SVL. Foot length 43% of SVL. Relative length of adpressed toes: IV>III >V >II >I. Largest discs on toes III and IV. Tarsal fold present, well defined, ending about three-fifths of the way along the distal end, not ending in a tubercle. Rudimentary web present between toes I–II, II–III and III–IV. Short flap along proximal border of fifth toe. Outer metatarsal tubercle oval, about twice longer than wide, slightly smaller than the rounded inner metatarsal tubercle; outer metatarsal tubercle elevated, subconical. Subarticular tubercles conspicuous, one each under toes I and II, two each under toes III and V, and three under toe IV. Supernumerary tubercles absent.

Coloration in life (based on holotype): dorsum pale to dark bronzy-brown, with an irregular dark brown pattern (Fig. 2). Dark canthal band running continuously across the tip of snout from eye to eye. Upper lips white, slightly pigmented by very small dark dots. Upper half of the tympanum dark brown; lower half of tympanum white, with pale brown border. Flanks dark, solid brown in dorsolateral border, marbled and paler in ventrolateral border. A pale inguinal stripe extending obliquely upwards to midflank, reaching the level of shoulder. Throat yellow. Collar unpigmented, without pale flecks or dots, but well defined between the yellow throat and the ventral coloration. Forelimbs and hindlimbs with well-defined dark brown dorsal crossbars on a paler brown background; dark crossing bars wider than pale ones. Ventral surface of limbs cream to yellow. Anterior half of venter dusted

Fig. 2. *Mannophryne venezuelensis* (Holotype EBRG 4924): a) dorsolateral view, b) ventral view; *M. trinitatis* (EBRG 4927) from Mount St. Benedict, Trinidad: c) dorsolateral view, d) ventral view.

with brown, slightly and diffusely pigmented with yellow on anterior margin, posterior half creamy-white. Palms and soles brown-pigmented.

Change of coloration in preservative fluid: in specimens preserved in 70% ethanol, the yellow pigmentation in venter and throat turns to white-cream; yellowish zones in ventral surfaces of limbs turn to pale grey and white-cream. Cooper and bronze tones turn to dark and pale brown, respectively. In males (EBRG 4920, 4921), vocal sac and two thirds of the anterior parts of the belly are grey-pigmented.

Variation within specimens in type series: morphometric variation in type series is shown in Table 2. Sexual dimorphism in size is evident. On average, males are smaller than females. One specimen (EBRG 4919) showed a thin pigmented collar. Of 29 specimens observed in the field (J. Manzanilla field-notes, 7 May 2002), 27 showed an unpigmented collar, the two remaining showing a paler pigmented collar.

Tadpoles

Three males carrying 7–10 tadpoles on their backs were observed on 7 May 2002. We examined 23 larvae (KU 181981) at Gosner's stage 24, from San Juan de Las Galdonas (Venezuela), altitude 180 m, close to the type locality of the species.

Tadpole body length 4.5–4.6 mm. Total length 12.6–13.5 mm. Body oval in dorsal view, depressed (wider than deep); snout rounded in dorsal view; dorsolateral nares, directed laterally; eyes situated and directed dorsolaterally; internarial distance wider than interorbital distance; chondrocranial elements scarcely visible through skin of head; spiracle sinistral, at about mid-length of body, opening below lateral midline; cloacal tube short, dextral opening; dorsal fin arising at body–tail juncture or on tail; caudal fins about equal in depth; caudal fins shallower than caudal musculature at mid-length of tail; caudal musculature weak, myomeres not well differentiated; caudal musculature extending to tip of tail; tip of tail acutely rounded (Fig. 3).

Mouth relatively small, directed anteroventrally, bearing weak lateral folds; a single row of papillae on margins of lips, except for a broad anterior diastema; beak weakly keratinized, with minute marginal serrations; keratinized portion of upper beak almost straight in relation to lower beak; lower beak V-shaped, smooth; two upper and three lower rows of denticles (Fig. 4); first upper rows complete, with largest denticles; second upper row widely interrupted medially; first lower row narrowly separated in the middle; second and third row complete; third lower row of denticles weakly keratinised.

In preservative fluid (10% formalin), dorsum and flanks are cream-coloured, skin of venter is transparent;

Table 2. Morphometric measurements (in mm) of the type series of *Mannophryne venezuelensis* sp. nov. and comparative measures for *M. trinitatis.* Measurements are presented as means, standard error (SE) in parentheses, and range; nad= no available data. For abbreviations, see text.

	Holotype EBRG-4924	Type series Females, *n*=9 Mean (SE) range	Type series Males, *n*=3 Mean (SE) range	Trinidad Females, *n*=9 Mean (SE) range	Trinidad Females, *n*=10 Mean (SE) range (La Marca, 1994)	Trinidad Males, *n*=3 Mean (SE) range	Trinidad Males, *n*=7 Mean (SE) range (La Marca, 1994)
SVL	22.1	21.5 (1.06) 19.2–23.0	19.4 (0.47) 18.9–20.0	23.6 (1.38) 22.0–24.3	23.4 (0.84) 21.6–24.7	21.9 (0.61) 21.2–22.1	21.1 (1.38) 20.1–24.4
HL	7.8	7.2 (0.74) 5.9–8.1	5.7 (0.79) 4.97–6.82	6.1 (0.43) 5.3–6.8	8.4 (0.33) 7.9–8.9	6.0 (0.03) 6.0–6.01	7.4 (0.31) 7.1–8.0
HW	8.1	7.8 (0.36) 7.3–8.4	7.1 (0.20) 6.8–7.3	8.5 (0.29) 8.1–9.0	nad	8.2 (0.31) 7.9–8.01	nad
SL	3.5	2.7 (0.62) 2.1–3.48	1.9 (0.11) 1.8–2.1	2.3 (0.17) 2.1–2.6	nad	2.4 (0.39) 2.0–2.8	nad
EN	2.1	2.1 (0.09) 2.1–2.2	1.7 (0.13) 1.6–1.9	2.1 (0.13) 1.9–2.3	2.2 (0.13) 2.0–2.4	2.0 (0.10) 1.89–2.0	2.0 (0.15)
IN	3.2	2.9 (0.22) 2.6–3.2	2.5 (0.32) 2.1–2.9	3.3 (0.08) 3.1–3.4	3.3 (0.12) 3.2–3.5	3.1 (0.1) 3.0–3.22	3.1 (0.11)
EYE	2.9	2.7 (0.12) 2.6–2.9	2.8 (0.17) 2.6–3.0	3.3 (0.15) 3.2–3.7	nad	3.22 (0.07) 3.2–3.3	nad
IO	2.6	2.7 (0.16) 2.5–3.0	2.4 (0.23) 2.2–2.7	2.8 (0.25) 2.4–3.2	nad	2.65 (0.03) 2.62–2.67	nad
TYM	1.5	1.39 (0.12) 1.2–1.6	1.2 (0.08) 1.1–1.3	1.5 (0.10) 1.4–1.7	nad	1.25 (0.15) 1.1–1.4	nad
FAL	4.3	5.07 (0.64) 4.3–6.1	4.7 (0.35) 4.4–5.2	5.1 (0.16) 4.8–5.3	nad	4.77 (0.47) 4.30–5.24	nad
HAND	5.5	5.5 (0.28) 5.2–6.2	5.3 (0.19) 5.0–5.4	6.1 (0.20) 5.9–6.5	5.9 (0.28) 5.3–6.3	5.69 (0.39) 5.3–6.1	5.4 (0.17) 5.2–5.7
TL	10.3	10.4 (0.65) 9.5–11.4	9.8 (0.36) 9.3–10.2	10.8 (0.32) 10.5–10.8	10.7 (0.33) 10.1–11.1	10.12 (0.24) 10.0–10.3	9.8 (0.22) 9.5–10.1
FL	9.4	9.8 (0.65) 9.4–11.3	8.9 (0.14) 8.8–9.11	10.0 (0.66) 8.5–10.7	10.0 (0.38) 9.5–10.5	10.0 (0.02) 10.0–10.1	5.4 (0.17)

dorsum and venter bearing dispersed melanophores; tail is cream-coloured, with higher concentration of melanophores on caudal musculature; fins translucent, with scattered melanophores on dorsal fin, almost absent on ventral fin.

Call characteristics

The advertisement calls of *Mannophryne venezuelensis* consist of a single, frequency-modulated note (Fig. 5, 2A,B) with different levels of intensity per note. Some notes show a similar intensity level throughout the note while other notes show up to three levels of intensity, marked by remarkable falls in intensity. Different note amplitudes are common within the same bout. Notes of the same amplitude have an average duration of 0.033 ms, with two separate amplitude levels; the first part has an average duration of 0.011 ms and the second an average duration of 0.22 ms, with the highest intensity peak located in the second part, and with three different amplitude levels: 0.011 ms, 0.021 ms and 0.013 ms; the

highest intensity is again at the second component of the note.

Otherwise, the advertisement calls of *Mannophryne trinitatis* consist of two frequency-modulated notes composed of paired pulses (Fig. 5, 1A,B) (average duration of the first pulse, 0.047 ms; second pulse, 0.053 ms; combined time of notes, 0.127 ms; average internote, 0.27 ms). The dominant frequency of the first pulse is lower than the second (first pulse average minimum frequency 3650.4 Hz, maximum 4544.7 Hz; second note average minimum frequency 3812.2 Hz, maximum 4749.3 Hz). The difference between dominant increasing frequencies per pulse is 894.3 Hz for the first pulse and 937.1 Hz for the second pulse. The difference of increasing frequency between both pulses is 163.8 Hz (first note) and 204.6 Hz (second note). Paired notes of *M. trinitatis* are very similar in intensity.

A comparison of call characteristics between *M. venezuelensis* and *M. trinitatis* suggests that the major difference between the two species is the single frequency-modulated note of *M. venezuelensis* versus the

Table 3. Summary of advertisement call characteristics of *M. trinitatis* and *M. venezuelensis.*

	M. venezuelensis	*M. trinitatis*
Duration between notes (S/N)	0.123 ms	0.075 ms
Number of notes per bout (N/NB)	15.4	19.4
Bout duration (BD)	4.8 s	3.1 s
Interbout duration (IBD)	1.88 s	5.5 s
Note duration (ND)	0.049 ms	0.073 ms
Minimum dominant frequency (mDF)	3731.3 Hz	3921.3 Hz
Maximum dominant frequency (MDF)	4647 Hz	5117.7 Hz
Difference between minimum and maximum dominant frequency (mMDF)	915.7 Hz	1196.4 Hz
Time of longest intensity rise (A)	0.023 ms	0.033 ms
Notes per second (N/S)	4 (double)	7

double pulsed frequency-modulated note of *M. trinitatis* (Fig. 5). Other likely constant differences in call characteristics of *M. venezuelensis* include shorter internote duration, shorter bout time duration, longer periods of time between bouts (>3 times) and greater note duration (Table 3). Other more variable, and therefore less distinctive, characteristics are: greater number of notes per bout, higher minimum and maximum dominant frequency, greater difference between minimum and maximum dominant frequency, increased duration of longest intensity and fewer number of notes per second (Table 3).

Chorus behaviour is different between the species. *M. venezuelensis* males synchronize their calls together into short call outbursts (from 26 analysed bouts: a mean average chorus call time of 5.5 s and mean intercall duration of 3.7 s). *M. trinitatis* males do not show this synchronization in calls. Instead, males call together for long periods of time with no particular breaks or apparent structure in composition of the calls. These long *M. trinitatis* choruses can be heard continuously on most afternoons during the wet season. Males of both species change colour (to black) when calling.

The advertisement call feature comparisons between *M. trinitatis* and *M. venezuelensis* reported here must be treated with caution for two main reasons. First, the advertisement calls are from representatives of each species from only one population and therefore may not represent the overall species call characteristics as different isolated populations may show intrapopulation call characteristics not reported here. Second, *M. trinitatis* bout length, number of notes per bout and interbout length, and *M. venezuelensis* amplitude, are very variable between and within individuals. This intrapopulation variation in call parameters may be dependent on physiological and social conditions of the frogs unaccounted

for in this study. Most of the data (double calls, number of notes per minute, duration of paired notes combined and individually, time between notes and minimum and maximum frequencies) presented here are in agreement with previous reports for *M. trinitatis* (Edward, 1974; Wells, 1980; Hardy, 1983). Minor differences between published calls and ours may be a result of population variability (Jowers, unpublished data), time of recording (time of day, month), recording equipment employed (other published *M. trinitatis* calls were recorded with tape recorders, less accurate in filtering calls versus our digital equipment) and analysis employed (type of software employed and accuracy of analysis).

Although temperature readings were not taken at the time of the recordings (intrapopulation variation in calls may be dependent on air temperature), several temperature readings (at 1 m above the ground) were taken the following year at the same sites (Venezuela and Trinidad) during the same month and hours of the day and the data suggest that these two populations were at similar temperatures during recordings, approximately 29 °C. Many hours of recordings analysed by MJJ of most known Northern and Central Range *M. trinitatis* populations and other newly described populations (Jowers & Downie, 2004) during two consecutive wet seasons, at differing temperatures (23–30 °C) and times of day (mornings and afternoons) have shown that this species has distinctive single and chorus call characteristics to the *M. venezuelensis* ones here reported. Further proof comes from observations (Jowers, unpublished) that females of
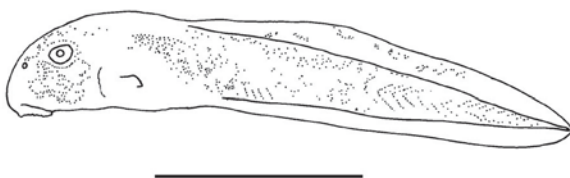
Fig. 3. Tadpole of *Mannophryne venezuelensis* (KU 181981). Lateral view. Scale bar = 5 mm.
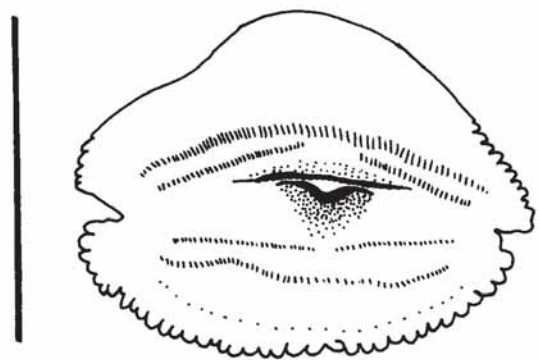
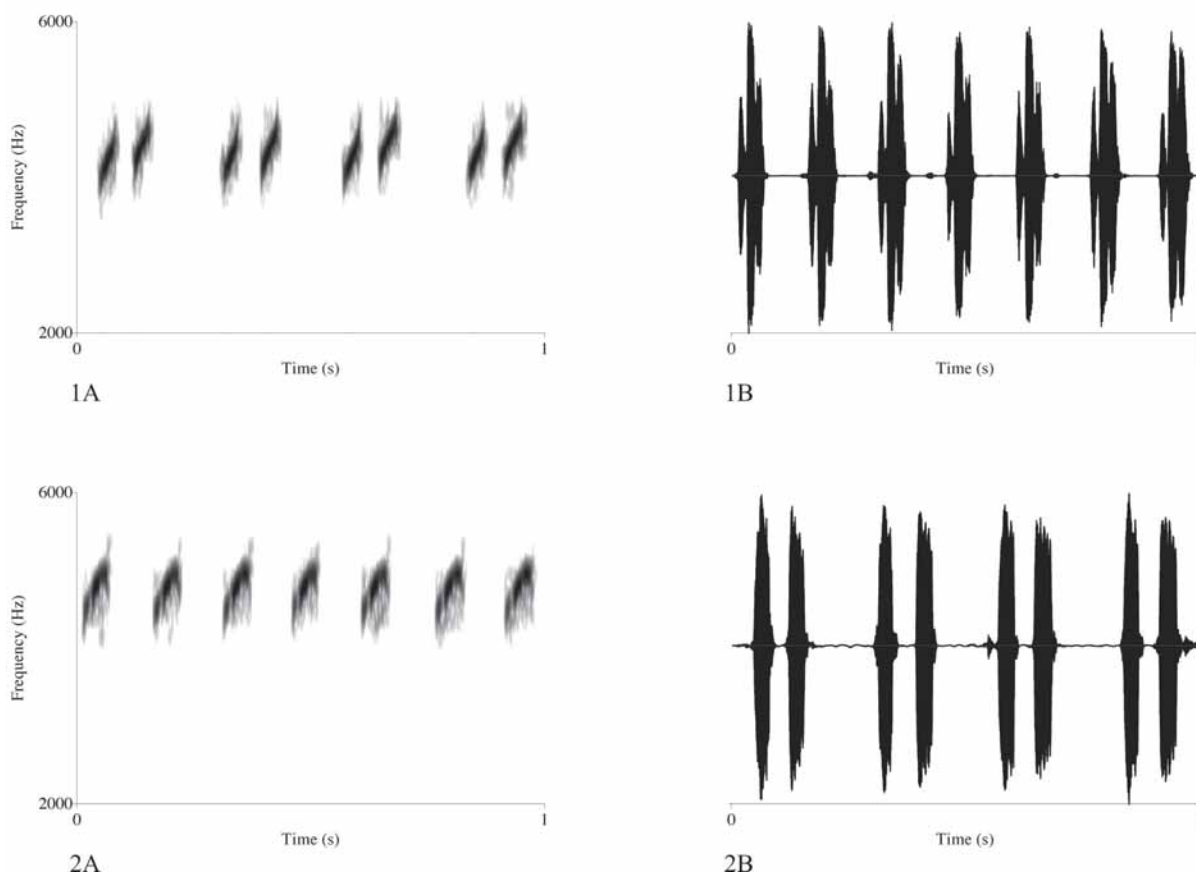**Fig. 4.** Tadpole of *Mannophryne venezuelensis* (KU 181981). Mouth, ventral view. Scale bar = 1 mm.

Fig. 5. Comparative spectrogram (left: 1A, 2A) and oscillogram (right: 1B, 2B) of the advertisement call of *M. trinitatis* (above) and *M. venezuelensis* (below).

M. trinitatis from Mount Saint Benedict (Trinidad) do not react to recorded advertisement call of continental males of *M. venezuelensis* but do react to their conspecific male calls.

Molecular data

Pairwise corrected distances (GTR) for the 16S dataset among populations of *M. trinitatis* (from the Northern and Central Ranges) do not exceed 0.43% sequence divergence. All 16S rDNA sequences of *M. venezuelensis* samples were identical. Divergence between *M. venezuelensis* and *M. trinitatis* populations range between 1.1 and 1.5%. Pairwise corrected distances (TMV+I+G) and uncorrected "p" distances between *M. venezuelensis* and *M. trinitatis* for the COI dataset range from 6.7 to 7.1%. Divergence within *M. trinitatis* ranges from 0.1 to 0.5%.

The 16S rDNA ML tree (-ln L=1268.4217) show that *Mannophryne* is monophyletic (bs ML: 87%; MP: 99%) (Fig. 6). Relationships among species of *Mannophryne* are poorly resolved, with *Mannophryne herminae* sister to the *M. venezuelensis–trinitatis* clade, and *M. collaris* basal to all of them. All analyses placed *M. trinitatis* as the sister group of *M. venezuelensis* (bs ML: 98%; MP: 100%). *Mannophryne trinitatis* is monophyletic (bs ML: 97%; MP: 94%). The MP analyses performed using the exhaustive search algorithm resulted in a single most parsimonious three (L=132: CI: 0.88; RI: 0.74; 104 variable characters, 40 phylogenetically informative), with a topol-ogy that differs from the ML tree in the relative position of *M. collaris*, sister to the *M. venezuelensis–trinitatis* clade (bs MP: 69%), and *M. herminae* basal to all of them.

Distribution

Mannophryne venezuelensis was found in and around mountain streams on the slopes of the Península de Paria, from near sea level to about 600 m; some specimens (EBRG 2549–2551, 2557) were in a stream that runs through a cocoa field. The geographic range occupied by this species is shown in Figure 7. *Mannophryne trinitatis* is restricted to the Northern and Central Ranges of Trinidad (Kenny, 1969, 1979; Murphy, 1997; Downie et al., 2001; Jowers & Downie, 2004).

The geographic range of the Venezuelan populations previously treated as *M. trinitatis* is now subdivided into two groups. One group includes the populations located in the Península de Paria described here as *M. venezuelensis*. A second group includes populations from the southern versant of the central Venezuelan Coastal Range and eastern Coastal Range (from Guárico to Sucre). The specimens of *Mannophryne* from this second group show affinities to *M. herminae*, but in agreement with La Marca (1992, 1994) we believe this hypothesis deserves further investigation. Given the morphological similarities among *M. herminae*, *M. venezuelensis* and *M. trinitatis*, we consider that the taxonomic status of the populations from the southern versant of the central Venezuelan Coastal Range should
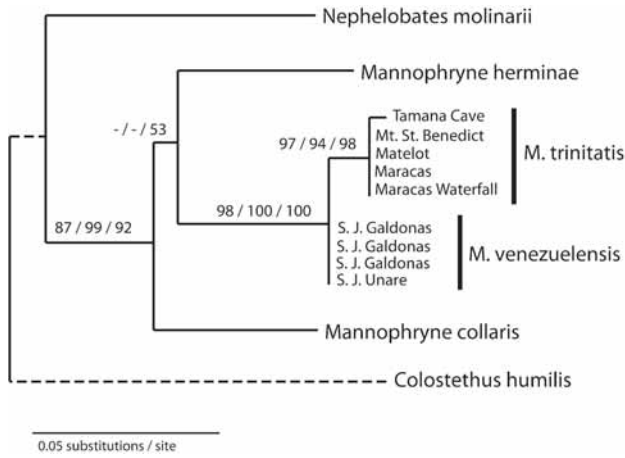
Fig. 6. Maximum Likelihood tree (-Ln=1268.4217) based on partial sequences of the mitochondrial gene 16S rDNA. Nonparametric bootstrap values (1000 pseudoreplicates) for Maximum Likelihood, Maximum Parsimony and Minimum Evolution are shown on the branches. Length of dashed lines does not correspond to real value.

be assessed by using molecular data (Manzanilla et al., in prep).

Biogeography

The regions inhabited by *M. venezuelensis* and *M. trinitatis* have been affected by complex and very dynamic orogenic processes. Three different scenarios – not mutually exclusive – can be proposed to account for the observed pattern of differentiation between *M. trinitatis* and *M. venezuelensis*.

1) The first hypothesis postulates that *M. trinitatis* and *M. venezuelensis* are vicariant species separated by the isolation of Trinidad from the Península de Paria mountain range. The Península de Paria range and the present islands of Trinidad and Tobago were part of a single geostructural unit until their uplift from the northern edge of the South American plate after colliding with the Caribbean plate (Ricardi, 1984; Fernández-Badillo, 2000). Although the exact time has not been ascertained, Trinidad was most probably part of the continent until the late Miocene (Liddle, 1946). According to this scenario, and assuming that no genetic exchange occurred by later land connections, the isolation of both taxa would date from no later than the late Miocene.

2) A second hypothesis involves the colonization of Trinidad by the ancestor of *M. trinitatis* through land-bridge connections between Trinidad and northeastern Venezuela. Murphy (1997) argued that Pleistocene climatic changes (causing sea-level drops of up to 150 m) could have resulted in land-bridge connections between Trinidad and northeastern Venezuela at least five to seven times within the last 140,000 years BP. However, because of the large amount of alluvial sediments transported by the Orinoco River into the Golfo de Paria and due to the fact that the Delta del Orinoco region is relatively recent (Pleistocene–Holocene), the sea depths between Trinidad and the continent, as well as the distance between

them are likely to be larger than estimated (the deltaic platform advances 54 m per year into the Golfo de Paria). Therefore, the colonization of Trinidad from the continental *Mannophryne* stock by land-bridge connections could only have occurred between the late Pleistocene and the Holocene period. Alternatively, if *M. trinitatis* was already established on the island prior to the formation of the land connections (first and second hypothesis), latter land bridges could have served as corridors for genetic exchange creating a misleading effect of recent differentiation.

3) The ancestor of *M. trinitatis* may have colonized Trinidad by rafting in river currents from adjacent continental areas, where the common ancestor of *M. trinitatis* and *venezuelensis* originated. This colonization pattern has been suggested for some reptiles from the coasts of Trinidad (e.g. *Chelus fimbriatus*, *Eunectes murinus*; Pritchard & Trebbau, 1984; Dixon & Michaud, 1992). The only area from where rafting is likely to occur between Venezuela and Trinidad is the Delta del Orinoco region. During the rainy season the water level of the Orinoco River increases considerably, transporting large portions of floating vegetation, capable of rafting to Trinidad. The salinity of the Golfo de Paria drops considerably during the rainy season as a consequence of the Orinoco River flow, increasing the possibility of amphibian survival on such rafts. Overseas dispersal by rafting has been previously proposed for amphibians (Vences et al., 2003b).

Rejection of the second hypothesis is based mainly on geographical grounds. Land-bridge formations took place far from Península de Paria, which is the only mountain region with potential habitat for *Mannophryne*. Because sea depths are relatively low near Icacos Point, in SW Trinidad, as well as along the Golfo de Paria inner sea, land bridges probably linked the 13 km that separates the southwestern region of the Icacos Point from Delta del Orinoco. However, land connections between Península de Paria and the northwestern mountain region of Trinidad were not possible during the Pleistocene, since the small islands located between these two regions
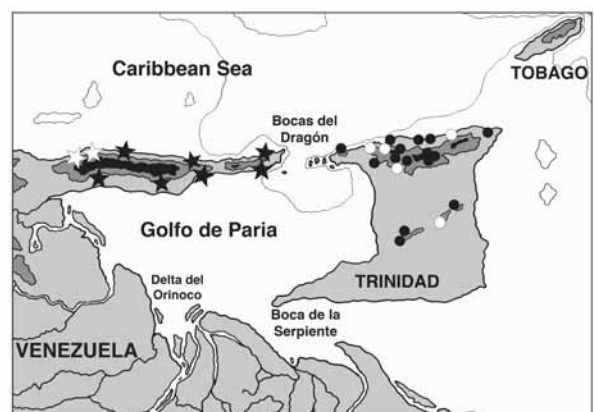

Fig. 7. Geographic distribution of *Mannophryne venezuelensis* (stars) and *M. trinitatis* (circles) based on museum and field note records. White symbols represent samples included in molecular analyses.

(Monos, Huevos, Chacachacare and Patos) are isolated by depths of nearly 250 m, deeper than the estimated 150 m Pleistocene sea drop (Murphy, 1997). Rejection of the third hypothesis is also on geographical grounds, since most of the rafts come from large river systems (such as the Orinoco drainage), where lack of hills would make the presence of *Mannophryne* species unlikely even during the cooler Pleistocene period.

On the basis of the COI estimates of 1.2–1.5% sequence divergence per Ma, the divergence between *M. trinitatis* and *M. venezuelensis* lineages occurred approximately 4.4–5.9 Ma, whereas 16S estimates suggest a divergence of 2.8–3.9 Ma. The molecular data at hand are thus not conclusive. Both age estimates, although they are not concordant, suggest that the differentiation might have occurred during the Pliocene, not giving support to the second and third hypotheses. Further analyses are still required since estimation of divergence dates among clades remains a difficult task in phylogenetic studies (Fromhage et al., 2004).

Conservation

Mannophryne species diversity appears to be widely underestimated (cf. La Marca, 1994; Mijares-Urrutia & Arends, 1999a,b), as has been the case for other genera of the Dendrobatidae, such as *Colostethus* (Caldwell & Lima, 2003) and *Nephelobates*. From a conservation perspective, this underestimation has a dramatic effect since conservation practices in Venezuela (MARN, 2001), as in most of the world, are focused at the species level. Undescribed, cryptic species are thus generally not included among specific conservation priorities. This is clearly the case of the *M. trinitatis* group. While *M. trinitatis* was thought to be a single taxon, its geographical range included a large territory in two countries (Trinidad & Tobago and Venezuela), and consequently no specific conservation measures were considered necessary for the taxon in Venezuela. After recognition of *M. venezuelensis*, *M. trinitatis* becomes endemic to Trinidad, while *M. venezuelensis* is restricted to the Península de Paria with independent conservation status.

Although a great part of the geographic distribution of *M. venezuelensis* lies within the Península de Paria National Park, this seems not to guarantee the conservation of the species. The old and traditional shift agriculture technique locally known as "conuco" is a frequent practice in the region (including within the National Park), and deforestation is increasing.

Direct conservation measures should focus on two aspects: forest preservation along stream edges and strict regulations for the use of agrochemical products in upstream coffee and cocoa plantations. Accurate and complete descriptions of the taxonomic diversity supported by population genetic analyses are being generated at a slower pace than the current rate of habitat destruction in many areas of Venezuela. As a consequence, large portions that are still unknown of the anuran biodiversity of the country may be disappearing before species can be described.

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