



Rediscovery and Mitochondrial Relationships of the Dendrobatid Frog *Colostethus humilis* Suggest Parallel Colonization of the Venezuelan Andes by Poison Frogs

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Abstract

Colostethus humilis Rivero, '1978' 1980 was rediscovered near the type locality. The species is redescribed, and data on its advertisement call are provided for the first time. Its morphology and colour pattern differ from other Andean dendrobatids. It shows morphological and bio-acoustic similarities to species of *Colostethus* from the Amazonian lowlands. A phylogenetic analysis of sequences of the mitochondrial 16S rRNA gene confirmed that *C. humilis* is related to Amazonian lowland *Colostethus*, rather than to other Andean poison frogs of the genera *Nephelobates* and *Mannophryne*. This indicates that several groups of basal dendrobatids independently have colonised the Venezuelan Andes.

Keywords: *Colostethus humilis*, Anura, Dendrobatidae, redescription, advertisement call, phylogeny, mitochondrial DNA, Venezuela.

Introduction

The Dendrobatidae are a family of frogs which are distributed in Central and South America and include colourful genera such as *Dendrobates* and *Phyllobates*, famous for their skin toxins. A species-rich basal subgroup of dendrobatids, however, shows cryptic coloration and general lack of skin toxins (e.g., Myers et al., 1991). These basal dendrobatids have long been subsumed in a single genus *Colostethus*. Recent work has shown the presence of differ-

ent lineages which are considered as separate genera, i.e., *Aromobates*, *Mannophryne* and *Nephelobates* (Myers et al., 1991; La Marca, 1992, 1994). All these three genera have been described from Venezuela and appear to be most common at higher altitudes in the Andes. In contrast, highest species diversity of the other dendrobatid genera is found in Neotropical lowlands both cis- and trans-Andean.

Dendrobatid systematics is in part difficult to assess (cf. Myers et al., 1991; Clough & Summers, 2000; Vences et al., 2000). One reason is the poor state of alpha-systematics of many basal taxa. Some recent contributions (e.g., Coloma, 1995) use standardised diagnoses and descriptions of *Colostethus* species, addressing also other than external characters (e.g., vocalisations, larval features). In comparison to this, most of the elderly species descriptions are of limited use. An example is *Colostethus humilis* Rivero, '1978' 1980, one of the least known dendrobatid frogs from Venezuela. Its description was based on five specimens collected near the town of Boconó, at an altitude of 1470m above sea level (Rivero, '1978' 1980).

During a recent survey of dendrobatid localities in the Venezuelan Andes, we rediscovered *C. humilis* above 2000 m elevation in the vicinity of the type locality. Despite its occurrence at this relatively high elevation, the collected specimen was phenetically different from other Venezuelan montane dendrobatids but showed obvious similarities to several lowland species of *Colostethus*.

In this paper we therefore investigate the relationships of *Colostethus humilis* to test whether the species is closely

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related to other montane dendrobatids or rather belongs to a group otherwise only known from lowland localities. For this purpose, we (1) redescribe the species based on the new material, (2) describe its advertisement call, and (3) assess its relationships to other dendrobatids using mitochondrial DNA sequences.

Material and methods

Coordinates of the study site were obtained from a Magellan 3000 GPS receiver. Altitude was assessed using a Casio hand altimeter chronograph. A single collected specimen of *Colostethus humilis* was fixed with 10% formalin after taking colour slides of coloration in life. Permanent conservation is in 70% ethanol at the collection of amphibians and reptiles of the Laboratorio de Biogeografía, Universidad de Los Andes, Mérida, Venezuela (ULABG) under the inventory number 3344.

Terminology and diagnostic characters and measurement definitions generally follow La Marca ('1996' 1997), with additions based on Grant et al. (1997) and Grant & Castro (1998). For the toe webbing formula of *C. humilis*, we follow the system of La Marca ('1996' 1997); to ease comparisons, we also provide, in parentheses, the webbing formula using the system of Savage & Heyer (1969, 1997) as modified by Myers & Duellman (1982). Measurements were taken, under a dissection microscope, with dial callipers to the nearest 0.1 mm.

Recording equipment consisted of a Sony WM-D6C tape recorder and a Vivanco EM 238 directional microphone. Air temperature at 1 m above ground was measured immediately after recording with a Greisinger GTH 175/MO digital thermometer. Recordings were digitised and analysed using a PC with Cool Edit 96 software (Syntrillium Software Co.). Sampling frequency was 32 kHz. Data in call descriptions are given as range (mean \pm standard deviation, and sample size n , in parentheses). Temporal call parameters are in milliseconds (ms). Terminology of call parameters analysed is after Heyer et al. (1990).

Muscle tissue samples of the dendrobatid species studied were preserved in 98% ethanol. DNA was extracted and a fragment of the mitochondrial 16S ribosomal RNA gene (up to 547 base pairs) was amplified and sequenced using protocols, primers and cycling procedures as described in Vences et al. (2000). Several hypervariable regions could not be reliably aligned and were therefore excluded from further analysis; altogether, 495 nucleotides were included in the analysis. Prior to phylogenetic reconstruction, we explored which substitution model fits our sequence data the best using the program MODELTEST (Posada & Crandall, 1998). Phylogenetic analyses were carried out using PAUP* (Swofford, 2001). We calculated maximum parsimony (MP) trees with gaps treated as a fifth character, and neighbor-joining (NJ) and maximum likelihood (ML) trees with gaps treated as missing data. In the MP analyses we conducted heuristic

searches with initial trees obtained by simple stepwise addition, followed by branch swapping using the TBR (tree bisection-reconnection) routine implemented in PAUP*. The NJ method was conducted under the LogDet model of character substitution. The ML trees were obtained using the substitution model proposed by MODELTEST for each data subset, respectively.

Beside five species already studied by Vences et al. (2000), namely *Phyllobates terribilis*, *Allobates femoralis*, *Colostethus bocagei*, *C. trilineatus* and *C. talamancae*, and the outgroup *Ceratophrys ornata* (Leptodactylidae), we included six Venezuelan dendrobatid species in the analysis. Voucher specimen numbers and EMBL/Genbank accession numbers of these are as follows: *Colostethus humilis* (ULABG 3344; AJ430673); *Mannophryne herminae* (La Entrada, Estado Aragua: ULABG uncatalogued; AJ430676); *Mannophryne collaris* (vicinity of Mérida, Estado Mérida: ULABG 4248; AJ430675); *Nephelobates molinari* (Las Playitas, Estado Mérida: ULABG 4502; AJ430678); *Nephelobates* sp. (near Mucuchies, Estado Mérida: ULABG 4496; AJ430677), *Dendrobates leucomelas* (Canaima, Estado Bolívar: toe clipped from a live specimen; AJ430674).

Results

Rediscovery of *Colostethus humilis*

The description of *Colostethus humilis* was based on five specimens collected in the Andes near the town of 'Boconó (Laguneta artificial del Ministerio de Agricultura) [Estado Trujillo], 1470 m' above sea level (Rivero, '1978' 1980). We did not locate this site with certainty, given that no establishment of this ministry appears to exist currently in or near Boconó. However, in the mornings of 15 and 16 June 1999, we heard several calling individuals (estimated to be about 15 to 20 specimens) of a small frog at km 1.8 on the unpaved road from Laguna Los Cedros to the Páramo de Guaramacal, Estado Trujillo, Venezuela (9°15'12"N, 70°13'07"W, ca. 2010 m above sea level). Calling specimens were hidden amongst vegetation on small slopes, in open grassland areas or under shrubs at the edge of cloud forest along the road. In contrast to many other *Colostethus* species, they were never found at water bodies but always at distances of at least 10–30 m from the closest (probably temporary) brook. Specimens were very difficult to locate and could not be observed in the moments of calling. In several cases, however, a *Colostethus* (Dendrobatidae), identified as *C. humilis* (see section on Variation, below), could be observed after intensively searching the sites from which calls were heard. Hence, there remains almost no doubt that the vocalisations heard (and recorded) correspond to this species (dendrobatids are usually diurnal and many species call away from water). No rain was recorded during our observations, but a high air humidity was perceived. No amphibians other than *C. humilis* were observed; only a small ground snake (*Atractus emigdioi*, ULABG 4473) was found under a rotten log.

This record is the second known of *C. humilis*, and extends its altitudinal range to 1470–2010 m above sea level. It lies in the same region as the type locality, in the vicinity of the town of Boconó (Estado Trujillo), on the E slope of the Venezuelan Andes, within a forest belt in the mountain region of Macizo de Guaramacal. The primary vegetation is a humid lower montane forest ('bosque muy húmedo montano bajo' in the classification of Ewel et al., 1976) located within 1500–2600 m of elevation. The formation of an atmospheric association giving rise to cloud forests is very common in this area. The heavy precipitation (2000–4000 mm annual mean) and relatively low temperatures (annual mean 12 to 18°C), along with the relatively low evapotranspiration (2 to 4 times lower than precipitation) create a rich humidity habitat. Due to the high amount of humidity, these environments are generally not suitable for agriculture, but we saw some use for pasture lands in areas sown with kikuyo grass (*Penisetum clandestinum*), a very competitive species introduced from cloud forest mountain environments in tropical Africa. Fortunately from a conservationist point of view, the new locality of *C. humilis* is located within the National Park General Cruz Carrillo, also known as Parque Nacional Guaramacal.

In the following, we provide a detailed redescription of *C. humilis* based on the new specimen collected. The original material of this extraordinary species of frog was not available for this study but it was briefly examined by the senior author several years ago.

Colostethus humilis Rivero, '1978' 1980 (Figs. 1–3)

Colostethus humilis Rivero, '1978' 1980: 105 'Boconó (Laguna artificial del Ministerio de Agricultura), E[sta]do. Trujillo, Venezuela, 1470 m'; Harding, 1983: 80; Frost, 1985: 90; La Marca, 1992: 27.

Diagnosis

A small *Colostethus* (known maximum SVL in adult males 21.8 mm) which can be distinguished from all other species of the genus by the following combination of characters: (1) skin of dorsum finely granular; (2) tympanum conspicuous, its horizontal length almost 50% that of eye; (3) tip of snout rounded in dorsal view, rounded and protuberant in lateral view; (4) canthus rostralis not well defined, more or less straight; (5) Finger I slightly shorter than Finger II; (6) disc on Finger III 1.6 times wider than precedent phalanx; (7) fingers with lateral keels; (8) cloacal sheath short with free broadly crenulate border; (9) tarsal fold conspicuous, with posterior end straight, not ending in tubercle nor thickened; (10) toe-web formulae: I2-III2-III1.5-1.5IV1-1.5V [I0-0⁺III1⁻-2III1⁺-3⁻IV3-1⁻V]; (11) toes with short lateral fringes; (12) dorsolateral bands absent; (13) disc on Toe IV 1.8 times wider than precedent phalanx; (14) a not well defined oblique pale lateral band, flanks dark; (15) high concentration of melanophores on chest, without forming a dark band; (16)



Fig. 1. Dorsolateral view of *Colostethus humilis* (ULABG 3344) in life.



Fig. 2. Ventral view of *Colostethus humilis* (ULABG 3344) in life.

ventrolateral band and black arm band absent; (17) venter dirty cream, without conspicuous pattern; (18) Finger III not swollen in males; (19) teeth short, not fang-like; (20) no median lingual process recognizable in the specimen studied.

Comparison with other species

Colostethus humilis cannot be confused with any other dendrobatid frog from Venezuela except *C. brunneus* (see

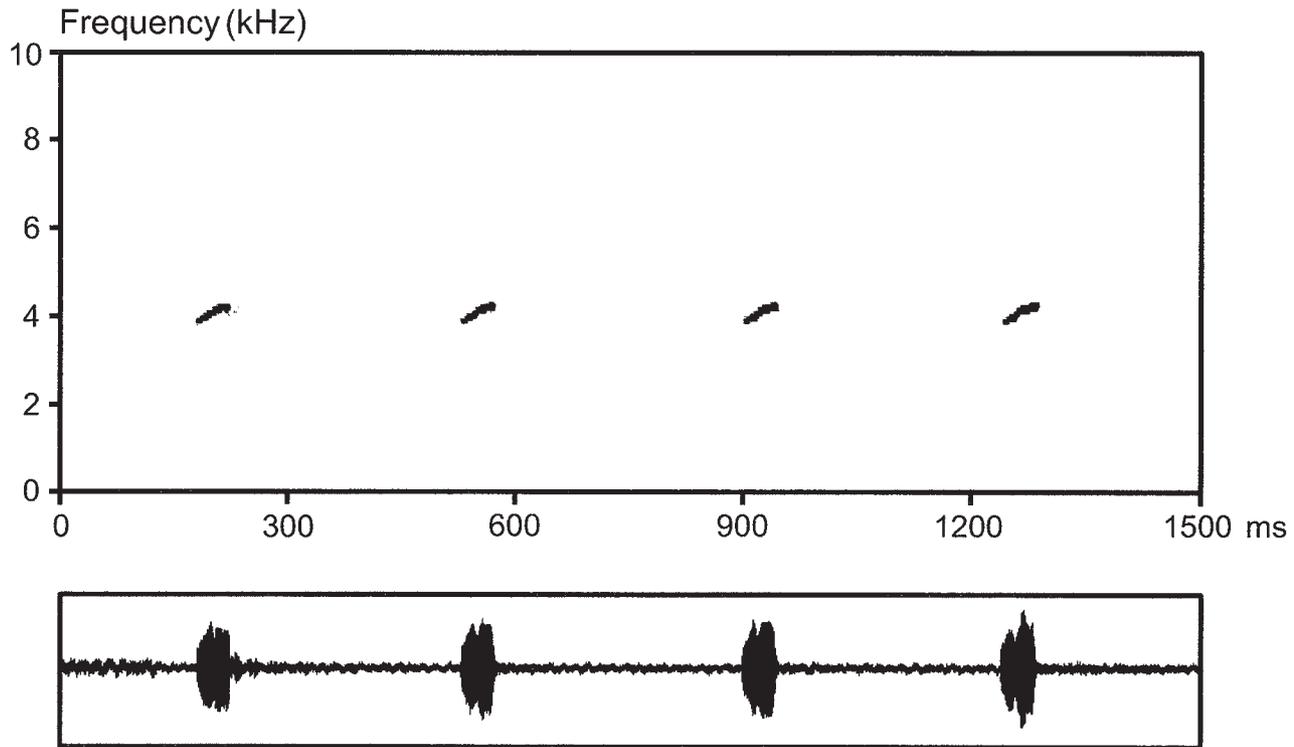


Fig. 3. Audiospectrogram and oscillogram (bottom) of the advertisement call of *Colostethus humilis* (ULABG 3344).

below). Its distinct coloration and small size allow easy differentiation from other species. Other Andean *Colostethus* from Venezuela do not have diamond blotches on dorsum in combination with dark flanks, characters also absent in *Mannophryne* (which have, besides, a distinct dark collar ventrally). Some *Nephelobates* may have dorsolateral dark bands, but all lack distinct dark flanks and possess a dermal flap above the vent.

Redescription

The following characters refer to ULABG 3344 (collected at the new locality described above), an adult male with white rounded testicles, about 1.4 mm in diameter, and vocal slits on both sides of the mouth floor. Snout-vent length 21.8 mm; tibia length 11.6 mm; head width 8.7 mm; head length 8.7 mm; tympanum width 1.6 mm; horizontal eye diameter 2.9 mm; inter-narial distance 3.2 mm; hand length 6.6 mm; foot length 11.4 mm. Head as wide as long. Interorbital region shagreened, slightly concave, without cranial crests. Interorbital distance about 1.5 times wider than the width of an upper eyelid. Canthus rostralis not well defined, more or less straight. Nostrils slightly protuberant, with narial opening directed laterally and slightly backwards. Nostrils closer to tip of snout than to anterior border of eye (about 1/3 distance between tip of snout and anterior border of eye). Loreal region almost vertical, descending abruptly to lips. Head, from above, sub ovoid. Tip of snout rounded in dorsal view, rounded and protuberant in lateral view. Horizontal

length on eye longer (approximately 1/3) than eye to nostril distance. Internarial distance longer (approximately 1/3) than eye to nostril distance. Tympanic membrane conspicuous, tympanic ring visible in its anterior and inferior parts. Tympanum separated from eye by a distance about 1/2 its horizontal length. Supratympanic region with a strong skin fold, more evident on left side of head, partially covering the superior and posterior part of tympanic membrane. An inconspicuous tubercle at rictus on left side of head, without a counterpart on right side; this tubercle is approximately 1/3 the width of the disc on Finger III.

Tongue broadly sub triangular, in outline, with its posterior end slightly wider (0.8%) than the maximum length. Posterior end of tongue broadly sinuous and concave. About 1/4 of posterior end of tongue not adherent to floor of mouth. Choanae small, rounded in outline, hidden by palatal shelf of maxillar arch. Maxilla and premaxilla bearing small, not fang-like, teeth.

Dorsum and upper eyelids finely granular, anterior part of head shagreened. Arms and forearms without tubercles. Outer metacarpal tubercle (palmar) entire, slightly subtriangular in shape, about 1.5 times longer than inner metacarpal tubercle (thenar). Thenar elongate, almost twice longer than wide. Supernumerary tubercles absent. Subarticular tubercles on fingers, rounded, except two on Finger I, being oval. Subarticular tubercles conspicuous, elevated. Discs on fingers slightly wider than long. Larger disc on Finger IV, approximately 1.3 times wider than precedent phalanx, and less than 1/3 the size of tympanum when disc is placed

directly against the later. Fingers without webbing. Lateral keels on Fingers II and III, more conspicuously on the internal and distal side of finger. Inconspicuous keel along the external margin of Finger IV. Finger I slightly shorter than Finger II. Finger III not swollen. Nuptial excrescences absent.

Cloacal opening above the medium line of thighs, covered above by a short sheath with a free and slightly crenulated border. Thighs, from above, smooth, although somehow shagreened in its distal portion near knee. Tibia length about 53% of snout to vent length. Tibiae without conspicuous tubercles. Tarsal fold conspicuous, with posterior end straight, not ending in tubercle and without thickening. Tarsal fold extending to medium part of tarsus, connected to lateral external keel on Toe I. Outer metatarsal tubercle rounded, slightly elevated, subconical, approximately twice the size of inner metatarsal tubercle. Inner metatarsal tubercle oval in shape, almost twice longer than wide. Subarticular tubercles on toes slightly oval, not elevated. Toes with a short webbing; toe-web formulae: I2-1II2-1III1.5-1.5IV1-1.5V [I0-0⁺II1-2III1⁺-3⁻IV3-1⁻V]. Toes with short lateral fringes. A conspicuous keel along external border of Toe V. Largest disc on Toe II, larger than any disc on fingers and remaining toes, almost doubling in width the precedent phalanx, slightly wider than long. Heels slightly overlap when thighs are held at right angles with body axis. When legs are adpressed forward, parallel to body, the heel reaches to anterior part of eye.

Coloration (in 70% ethanol after fixation in 10% formalin) of dorsum uniformly brown, with some large albeit not conspicuous blotches, slightly paler on head and posterior part of back. Extremities, above, with dark brown bars intercalated with pale grey areas with dark brown melanophores. Thighs, below, pale brown. Palms and soles darkened. Fingers and toes with dorsal bars. Dermal scutes on digits, dark grey. Flanks greyish, with a fine brownish stippling. A kind of pale band, not well defined, from groin to almost the insertion of arm. Black canthal band, slightly darker than top of head. Lips grey, with brown stippling, in a pattern that extends backwards covering tympanum and reaching to insertion of arm. Tympanic membrane with a larger density of melanophores than anywhere else on this grey pattern. Tympanic annulus cream, as seeing through skin. Ventral surfaces dirty cream (by profusion of melanophores). A large concentration of melanophores on posterior part of throat, without forming a 'collar' pattern.

In life (according to hand written field notes and colour slides by the authors), the dorsal ground colour was light brown, dorsally with three diamond-shaped darker areas having irregular olive flecks and bordered by an olive line. The lateral side was dark brown with whitish spots at posterior region; ventrolateral region whitish. One small dark brown bracelet occurred on femur and tibiofibula; rest of leg and arm as well as feet and hand were irregularly patterned with dark brown flecks (occasionally with whitish spots included). The labial line was pinkish white, the iris golden.

Ventrally with reduced pigmentation, the belly was whitish, the chest and extremities beige. The throat was black, although anteriorly grey.

Variation

We consider ULABG 3344 as *Colostethus humilis*, based on its small size and distinct coloration. No other Venezuelan species in the genus is known to bear a combination of diamond-shape blotches on the dorsum, a dark band on flanks, white venter and absence of dorsolateral and ventrolateral pale bands. The specimen collected by us is larger than the holotype. According to the original description (Rivero, '1978' 1980), the latter is a female with SVL 14.0 mm (of unknown reproductive condition, but possibly subadult considering the small size). The larger size of the adult male described here suggests that *C. humilis* may reach beyond 22 mm. ULABG 3344 differs from the holotype (characters of this given within parentheses) in a few minor traits which may reflect different state of preservation, sexual condition or individual variation, or partly a difference in subjective appreciation of the characters: having a more webbed foot (according to the text and following the figure in the original description, the toe web formula of the holotype could be stated as: I1-0.5II1.5-1.5III0-0IV0-0V), tympanum conspicuous (not apparent), Finger I slightly shorter than Finger II (equal in length), about 1/4 of posterior end of tongue not adherent to floor of mouth (1/2 free), canthus rostralis not well defined, more or less straight (angular, somewhat curved), tarsal fold conspicuous, with posterior end straight, not ending in tubercle and without thickening (limited to an oblique tubercle placed about 1/3 the distance between heel and ankle), heels slightly overlap when thighs are held at right angles with body axis (heels touch but do not overlap), dorsum finely granular (smooth).

Advertisement call

Due to the repeated emission of the same type of call from the same site, we consider the kind of vocalisations heard as advertisement calls (see Duellman & Trueb, 1986). These (recorded from a single non-collected individual on 15 June 1999, ca. 11:30h at 19.5°C air temperature; Fig. 3) can be described as series of 7–16 (10 ± 3 , $n = 9$) intense metallic 'click' notes. Note series were repeated after irregular intervals of several seconds. Each note had a duration of 39–53 ms (47 ± 3 ms, $n = 53$). Duration of intervals between notes was 293–906 ms (428 ± 134 ms, $n = 51$). Intensity of notes generally increased during the first half of each call; the last 2–3 notes generally decreased again in intensity. Especially in the most intense notes of a call, it was possible to observe two or three distinct intensity maximums (pulses). Note repetition rate was 1.8–2.5/s. Frequency was between 3700 and 4400 Hz. Notes were slightly frequency-modulated, with an initial dominant frequency at about

3900 Hz increasing to a dominant frequency of 4200 Hz at the end of a note.

Molecular phylogeny

Of the 495 characters included in the analysis, 346 were constant, 70 variable but parsimony-uninformative, and 79 parsimony-informative. Maximum parsimony searches recovered a single most parsimonious tree (284 steps; consistency index 0.665, retention index 0.550). All three tree reconstruction methods recovered four major clades among the species included in the analysis: (1) a lineage containing the two aposematic species (*Phyllobates* and *Dendrobates*) and *Colostethus bocagei*; (2) a lineage containing the two species of *Mannophryne*; (3) a lineage containing the two species of *Nephelobates*; (4) a lineage containing *Allobates* and the three remaining *Colostethus* studied including *C. humilis*.

Bootstrap analyses resulted in significant support for these lineages, the trees differed in the arrangement of them relative to each other; neighbor-joining (not shown) arranged *Nephelobates* as sister group of *Mannophryne*, while maximum likelihood (Fig. 3) and maximum parsimony (not shown) arranged *Nephelobates* as sister group of the clade containing *Allobates*, *Colostethus humilis*, *C. trilineatus* and *C. talamancae*. However, all results agreed in placing *Colostethus humilis* as close relative of *Allobates*, *C. trilineatus* and *C. talamancae*. The general topology totally agrees with that of Vences et al. (2000) who studied six of the 12 dendrobatid species included here; the grouping of *Colostethus bocagei* with aposematic dendrobatids (rather than with *Colostethus trilineatus* and *C. talamancae*) was also apparent from the cladograms of these authors.

Discussion

In the original description, *C. humilis* was considered close to the group of *C. brunneus* as originally described, and to *C. marchesianus* (both from the Amazon basin and peripheral areas), based on its small size and shortness of pedal membrane (Rivero, '1978' 1980). Furthermore, it shares with *C. brunneus* the presence of diamond shaped marks on the dorsum.

The advertisement call of *C. humilis* shows some similarities to those of *C. brunneus* from Bolivia sensu Gonzales et al. (1999) (Lötters et al., in press), *Colostethus* sp. cf. *marchesianus* from Ecuador (Coloma, 1995 and pers. comm.) as well as *C. trilineatus* and *Colostethus* sp. (aff. *trilineatus*) from Bolivia (Köhler & Lötters, 1999). The longer note length and the lower note repetition rate may be specific characters of *C. humilis*, but are possibly also explained by the relatively low temperature at time of recording. Nevertheless, we agree with Lötters and Kneller (2000) who raised doubts about the relevance of call characteristics to access phylogenetic relationships in dendrobatid frogs.

In a major classification of *Colostethus* species, Rivero ('1988' 1990) placed *C. humilis* in a category of 'doubtful species'. *Colostethus brunneus*, *C. marchesianus* (i.e., populations of uncertain taxonomic status formerly included in this species which do not have Finger III swollen in males (versus Finger III swollen in *C. marchesianus* sensu stricto); Morales, 1994; L.A. Coloma, pers. comm.) and *C. trilineatus* [including a *Colostethus* from Bolivia (which is conspecific with *C. trilineatus*, according to Gonzales et al., 1999), *C. kingsburyi*, *C. olfersoides*, *C. peruvianus* and *C. ranoides*] were classified in the Amazonian *brunneus* group (group II) of the eight groups erected in that paper. Rivero and Serna ('1988' 1989) subsequently added the trans-Andean *C. talamancae* to this group and, in addition, erected a ninth group. Species groups suggested by Rivero ('1988' 1990) and Rivero and Serna ('1988' 1989) have been discussed controversially; currently, they are considered as phenetic assemblages (e.g., Myers et al., 1991; Coloma, 1995). Summarising, *C. humilis* shows similarities in advertisement call and morphology (see above) to some members of the *brunneus* group of Rivero ('1988' 1990). This clearly corresponds to its molecular affinities to *C. talamancae* and *C. trilineatus* which both have been referred to this phenetic group.

Phylogenetic relationships of basal dendrobatids are poorly known (Myers et al., 1991; Coloma, 1995; Vences et al., 2000). Among them, in the Venezuela Andes, at least three lineages can be distinguished: (1) the enigmatic *Aromobates nocturnus*, (2) cloud forest members of the genus *Nephelobates*, and (3) the 'collared' frogs of the genus *Mannophryne* (which mainly occur at lower elevations). Additionally, a number of species of unknown affinities are known which currently are referred to the genus *Colostethus*.

The phylogenetic positions of *Aromobates* and most *Colostethus* spp. are unknown. In our molecular trees, the two included species of *Nephelobates* and *Mannophryne* respectively form monophyletic groups, which provides support for their separate status. However, we could significantly exclude that *Colostethus humilis* forms part of either of these Andean lineages. Although, we do not have molecular data for *Aromobates nocturnus*, the large morphological differences between this species and *C. humilis* (cf. Myers et al., 1991) makes phylogenetic relationships between them highly improbable. In contrast, our molecular data placed *C. humilis* with high support in a lineage containing two *Colostethus* and one *Allobates* distributed in the Amazonian lowlands (Fig. 4). This conclusion also seems to be in agreement with the phenetic data on morphology, coloration and advertisement calls as discussed above. Other molecular data (Clough & Summers, 2000) indicate that at least one other lowland species, *C. marchesianus* sensu lato, also is closely related to *Allobates femoralis* and therefore probably belongs to this lineage. The available data indicate that the origin of *C. humilis* in the Andes was phylogenetically independent from the largely montane genera *Aromobates*, *Mannophryne*

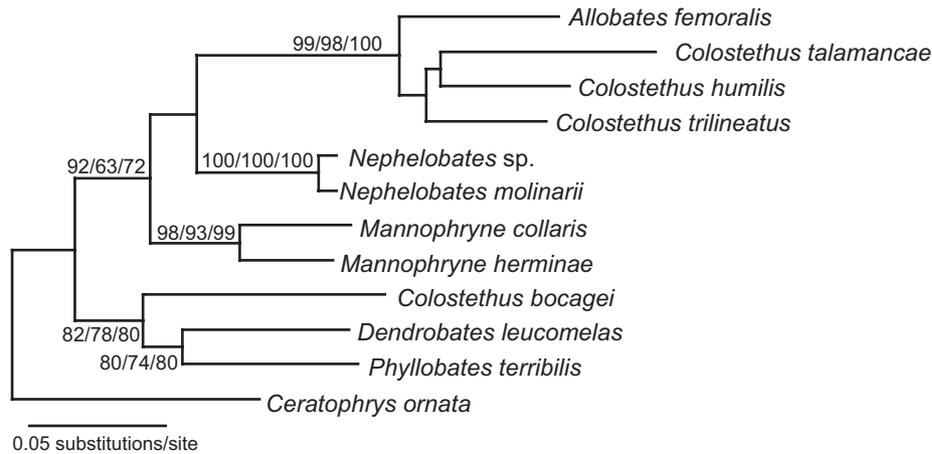


Fig. 4. Maximum likelihood (ML) phylogram based on analysis of sequences of the mitochondrial 16S rRNA gene (495 nucleotides) in 11 species of dendrobatids. The numbers are bootstrap values in percent of maximum likelihood, maximum parsimony and neighbour-joining analyses (100, 2000 and 2000 replications).

and *Nephelobates*, and probably due to an independent colonization event by its lowland ancestors.

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