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ORIGINAL ARTICLE

The Golden Tree Frog of Trinidad, *Phyllodytes auratus* (Anura: Hylidae): systematic and conservation status

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Analyses of mitochondrial 12*S* and 16*S* rDNA sequences lead us to propose that the Neotropical hylid genus *Phyllodytes* is paraphyletic. The level of divergence between the Trinidadian endemic *P. auratus* and the two Brazilian *Phyllodytes* included in the analyses (*Phyllodytes* sp. and *P. luteolus*) is greater than that of inter-generic distances within the Lophiohylini. The molecular evidence here reported, behavioural differences and restriction to a single host plant in a geographically limited area differentiates *Phyllodytes auratu*s from other supposed *Phyllodytes* evidencing its unique taxonomic status. We therefore propose a new genus, *Phytotriades* gen. nov., for *Phyllodytes auratus. P. auratus* mitochondrial cytochrome *b* sequences obtained from one locality (seven individuals) contained two haplotypes, one individual differing by a single transition. The other locality (one individual) had the commoner haplotype. The low genetic divergence between the two populations suggests recent isolation at these two localities.

Keywords: DNA; Golden Tree Frog; *Phyllodytes auratus*; *Phytotriades* gen. nov.; Trinidad

Introduction

Phyllodytes auratus (Boulenger, 1917), the Golden Tree Frog of Trinidad, has been reported from the two highest peaks of the island's Northern Range, El Tucuche and Cerro del Aripo. Kenny (1969) found this species only on El Tucuche at elevations higher than 600 m above sea level and put forward evidence, from his own observations, suggesting that it was absent from four other adjacent peaks with adequate altitude. However, Read (1982) was first to find the frog on a second mountain, Cerro del Aripo. Murphy's (1997) review of the amphibians of Trinidad reported *Phyllodytes auratus* from El Tucuche, Cerro del Aripo "and probably Morne Bleu Ridge". Clarke et al. (1995), in a paper not known to Murphy (J. Murphy, personal communication), recorded the Golden Tree Frog only on El Tucuche and Cerro del Aripo. They surveyed two other mountains, Morne Bleu Ridge and Chaguaramal, but both lacked the specific vegetation apparently required by the frog, though Kenny (1969) had found suitable vegetation on these peaks 30 or so years earlier.

The vegetation required by *Phyllodytes auratus* is the epiphytic giant tank bromeliad *Glomeropitcairnia erectiflora*. All reported sightings of the frog have been from on or within this plant, which can hold considerable quantities of water. Clarke et al. (1995) opened 27 *G. erectiflora* on El Tucuche and found a mean water content of nearly 700 ml. Six of these contained Golden Tree Frog adults and/or tadpoles. At Cerro del Aripo, of 13 *G. erectiflora* opened over 2 years, six contained Golden Tree Frog adults and/or tadpoles (Clarke et al. 1995).

The Golden Tree Frog's requirement for bromeliads containing large volumes of water presumably relates to the frog's reproductive mode. Clutch size is unknown but there is evidence that it is small. Kenny (1969) reported a maximum of five tadpoles per bromeliad tank and Clarke et al. (1995) reported a maximum of six. S. Rudd (personal communication) found a clump of four eggs stuck to a leaf by their jelly coats. On hatching, tadpoles are 14 mm long (J.R. Downie, unpublished data). They grow to a maximum length of 40 mm (Kenny 1969), which may take some time given the limited resources available in a bromeliad tank.

The Global Amphibian Assessment (GAA) (Hardy 2004) rated *Phyllodytes auratus* as Critically Endangered on the basis of its highly restricted range, but also because of "continuing decline in extent and quality of its habitat". Although the GAA was unaware of any conservation measures, the frog's main location, El Tucuche, is both part of a game

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sanctuary and a prohibited area where access requires government permission (N. N-Gyan, personal communication): however, it would be fair to say that policing access to the mountain is not easy.

Given that the Golden Tree Frog now exists as two small nearby isolated populations (El Tucuche and Cerro del Aripo are about 15 km apart), we expected that these are remnants of a recently larger population and thus to find low genetic divergence between the two populations.

Another aim of the work reported here was an assessment of the phylogenetic status of the Golden Tree Frog. The species is placed in the genus *Phyllodytes*. The genus currently contains 12 species, divided into four species groups, on the basis of color patterns (Peixoto et al. 2003; Caramaschi et al. 2004; Caramaschi & Peixoto 2004). Apart from *P. auratus*, all occur in Brazil (Caramaschi & Peixoto 2004; Caramaschi et al. 2004; Cruz et al. 2006). Based on the unusual generic biogeography (to the best of our knowledge there is no other genus within the family Hylidae with a similar distribution) and *P. auratus*'s morphological and behavioral differences from all other *Phyllodytes* species, we hypothesized that current classification of *P. auratus* is likely to be erroneous. In this paper we assessed the relationships of *P. auratus* using a molecular phylogenetic approach which has proved to be a useful tool in anuran taxonomy (Faivovich et al. 2005; Manzanilla et al. 2007).

Hitherto, the assemblage of these taxa into a single genus has not been based upon either well-established morphological synapomorphies or molecular divergence, but upon variable characters that could prove to be unreliable, such as bromeliad use and the presence of odontoids (''fangs'') on the mandible, nowadays known as a homoplasious character subject to sexual selection (Fabrezi & Emerson 2003). *Phyllodytes auratus* is distinguished from other *Phyllodytes* by its restriction to cloud forest woodland sites above 600 m elevation, by the unique presence of longitudinal colored dorsal stripes (Bokermann 1968) and by its apparent lack of vocalization. Clarke et al. (1995) and Read (1982) reported spending several nights in total in the Golden Tree Frog's habitat without hearing it call.

Faivovich et al.'s (2005) review of the Hylidae followed Caramaschi et al. (2004) in recognizing *Phyllodytes* as a single taxonomic unit, but they were able to include in their molecular analysis only two Brazilian species, *P. luteolus* and *Phyllodytes* sp. Since their sample did not include *P. auratus* they were unable to provide a molecular test of monophyly.

In this paper, in addition to molecular analyses using mitochondrial rRNA markers of samples from the two Trinidad locations of *P. auratus*, we provide a comparison with 10 genera within the Lophiohylini (Faivovich et al. 2005) including the two Brazilian *Phyllodytes* species used in Faivovich et al. (2005).

Materials and methods

Study sites and specimen collection

Phyllodytes auratus was collected in August 2003 from leaf axils of tree-growing bromeliads (*Glomeropitcairnia erectiflora*) approximately 1–4 m above the ground, near the summits of El Tucuche (937 m above sea level) and Cerro del Aripo (940 m above sea level) (Figure 1). Sampling was done around the summit of El Tucuche (within 30 m from the 150 m^2 cleared area), for 2 h. Because we did not have authorization to cut down *G. erectiflora* at any of the sites, sampling had to be carried out by gently pulling back the bromeliad leaves. When disturbed, *P. auratus* adults leaped out of the plants and were then hand caught. Seven adults were caught from ~20 *G. erectiflora* at El Tucuche and we took toe-clips from them. Cerro del Aripo was accessed through the Morne Bleu Ridge, but no *G. erectiflora* or *P. auratus* were seen there. Most bromeliads growing within 4 m from the ground were sampled (~20) at the summit of Cerro del Aripo or in the surrounding area but only one *P. auratus* tadpole was found after a 2 h search and a clip of its tail was taken. Tissue samples were preserved immediately

Figure 1. Map of Trinidad with the localities where *Phytotriades auratus* comb. nov. (circles) and *Glomeropitcairnia erectiflora* are found. A, El Tucuche; B, Cerro del Aripo; C, Chaguaramal. Gray areas represent hill ranges.

in 95% ethanol. All animals were released after sampling.

DNA studies

Total DNA was extracted and purified using standard phenol/chloroform protocols (Sambrook et al. 1989). Excised bands and PCR product were recovered using a QIAquick™ Gel Extraction Kit. The oligonucleotide primers used for amplification and sequencing are shown in Table 1. Polymerase chain reactions (PCR) were performed with commercial reagents and recommended reaction mix concentrations (Promega, Glasgow, UK). Sequencing of both strands was performed using fluorescent dideoxy chain terminators (BigDyes, PE Biosystems) and analysed in an ABI 377 gel electrophoresis apparatus (Applied Biosystems, Perkin Elmer).

Sequences were aligned and edited in Seqapp 1.9a (Gilbert 1993). Terminal amplification primers were excluded from the alignment and two alignments were prepared.

12*S* and 16*S* rDNA fragments from 10 genera within the Lophiohylini (see Table 2) were obtained from GenBank (www.ncbi.nlm.nih.gov), concatenated and aligned, using Clustal-X 1.81 (Thompson et al. 1997) with 10/10 open/extend gap penalties and minor modifications by eye. The alignment of sequences (including specimen field note number T521) followed *Xenopus laevis* secondary structure models (www.rna.icmb.utexas.edu) to make decisions about ambiguous regions. After exclusion of 104 sites whose alignment was potentially ambiguous this alignment of 1170 nucleotide sites (728 nt 12*S* rDNA; 442 nt 16*S* rDNA) was used to examine the relationships of *Phyllodytes* within Lophiohylini.

Because the 12*S* and 16*S* amplified segments from different *P. auratus* individuals were almost invariant, within-species variation was assessed using a 390 nt fragment of the faster-evolving cytochrome *b* gene (cyt *b*). The sequences obtained could be aligned without indels and showed an open reading frame when translated with the vertebrate mitochondrial genetic code.

All systematic analyses were performed with PAUP*4.b.10 (Swofford 2002) or MrBayes 3.0 (Huelsenbeck & Ronquist 1999). Heuristic search of 1000 pseudoreplicates was used to test for the presence of non-random structure in the data (PTP Test; Faith & Cranston 1991) and for incongruence of rDNA data partitions (Partition Homogeneity Test; Farris et al. 1995).

Trees were constructed by maximum likelihood (ML) and Bayesian maximum likelihood (BML) optimality criteria. Clade support in BML was obtained by the frequency with which a clade appeared in the saved trees. The best-fit ML model under the Akaike information criterion (Posada & Buckley 2004) was identified using MrModelTest 2.0 (Nylander 2004) and Modeltest (Posada & Crandall 1998) for the ML tree construction.

MrBayes was used with default priors and Markov chain settings, and with random starting trees. The gamma shape parameter and the proportion of invariant sites were estimated from the data. Trees were sampled every 100 generations for 1,500,000 generations. The log-likelihood scores plateau was reached at 30,000 generations. A consensus tree was constructed from the last 5000 trees (500,000 generations). Relative rate tests were performed with RRtree 1.1.13 (Robinson et al. 1998). Saturation of substitutions was evaluated by plotting (in Excel) transition against transversion '*p*' distances. The linear or power regression with the highest r^2 value was identified as the best-fitting one.

Results and discussion

The concatenated rDNA alignment base composition was not heterogeneous (χ^2 tests, $P=1$) and there was strong non-random structure (PTP tests, $P=0.01$). Inclusion of *Trachycephalus jordani* (used in Faivovich et al. 2005) in the analyses significantly increased the heterogeneity between rDNA partitions. Exclusion of

Table 1. Primers used to amplify and sequence cyt *b*, 12*S* and 16*S* rDNA.

Primers	Gene	Sequence	Reference
L15172	cyt *b*	5′-TGAGGACAAATATCATTCTGAGG-3′	Hillis et al. (1996)
H15557	cyt *b*	5′-GGCGAATAGGAARTATCATTC-3′	Hillis et al. (1996)
L617	12*S* rDNA (I, II domain)	5′-CAAAGCAYAGCACTGAAGATG-3′	Feller and Hedges (1998)
H1066	12*S* rDNA (I, II domain)	5′-GCATAGTGGGGTATCTAATCCCAGYYYG-3′	Feller and Hedges (1998)
L1091	12*S* rDNA (III domain)	5′-AAAAAGCTTCAAACTGGGATTAGATACCCCACTAT-3′	Kocher et al. (1989)
H1478a	12*S* rDNA (III domain)	5′-CYCTGACGGGCGRTDTGT-3′	Kocher et al. (1989)

Note: *Phyllodytes auratus* 12*S* rDNA was amplified with two pairs of primers resulting in two non-overlapping fragments while all other 12*S* rDNA sequences included in the alignment did not lack this region. Thus, other 12*S* rDNA sequences had ca. 30 extra sites that corresponded to the non-sequenced region between the two *P. auratus* 12*S* rDNA non-overlapping fragments. This region was excluded from the alignments.

Table 2. List of specimens examined, localities, genes sequenced, voucher numbers, *Phytotriades auratus* comb. nov. specimen field note numbers and GenBank accession numbers.

Taxa	Locality	Vouchers or field numbers	Genes	GenBank accession
Hylini				
Smilisca phaeota	Captive raised	Rds 786	12*S* and 16*S* rDNA	AY843764
Anotheca spinosa	Mexico: Oaxaca, Ixtlan de Juarez	ENS 10034	12*S* and 16*S* rDNA	AY843566
Triprion petasatus	Belize: Hummingway Hwy	Rds 749	12*S* and 16*S* rDNA	AY843774
Hyla versicolor	Venezuela: Bolivar: Cerro Guanay	UMFS5545	12*S* and 16*S* rDNA	AY843682
Isthmohyla rivularis	Costa Rica: Heredia	MVZ 149750	12*S* and 16*S* rDNA	AY843659
Lophiohylini				
Trachycephalus venulosus	Guyana: Dubulay Ranch	AMNH-A 141142	12*S* and 16*S* rDNA	AY549362
T. mesophaeus	Brazil: Rio de Janeiro	CFBH 5780	12*S* and 16*S* rDNA	AY843718
T. resinifictrix	Venezuela: Amazonas	AMNH-A 131201	12*S* and 16*S* rDNA	AY843719
T. hadroceps	French Guyana: Kaw Road	MNHNP 2001.0814	12*S* and 16*S* rDNA	AY843717
T. nigromaculatus	Brazil: Espirito Santo	N/A	12*S* and 16*S* rDNA	AY843772
Osteopilus septentrionalis	Cuba: Guantanamo	VSNM 317830	12*S* and 16*S* rDNA	AY843712
O. vastus	Pet trade	AMNH A-168415	12*S* and 16*S* rDNA	AY843713
O. crucialis	Jamaica: Manchester Parish	N/A	12*S* and 16*S* rDNA	AY843710
O. dominicensis	Pet trade	AMNH A-168210	12*S* and 16*S* rDNA	AY843711
Osteocephalus cabrerai	Brazil: Acre	LSUMZ H-13720	12*S* and 16*S* rDNA	AY843705
O. oophagus	French Guyana: Kaw Road	MNHNP 2001.0828	12*S* and 16*S* rDNA	AY843708
O. leprieurii	Venezuela: Amazonas	AMNH A-131254	12*S* and 16*S* rDNA	AY549361
O. taurinus	Venezuela: Amazonas	AMNH A-131245	12*S* and 16*S* rDNA	AY843709
Itapotihyla langsdorffii	Argentina: Misiones	MACN 38643	12*S* and 16*S* rDNA	AY843706
Nyctimantis rugiceps	Ecuador: Napo	N/A	12*S* and 16*S* rDNA	AY843780
Aparasphenodon brunoi	Brazil: Espirito Santo	CFBH 2715	12*S* and 16*S* rDNA	AY843567
Corythomantis greeningi	Brazil: Alagoas	CFBH 2968	12*S* and 16*S* rDNA	AY843578
Argenteohyla siemersi	Argentina: Corrientes	MACN 38644	12*S* and 16*S* rDNA	AY843570
Tepuihyla edelcae	Venezuela: Estado Bolivar	MNHNP 1998-311	12*S* and 16*S* rDNA	AY843770
Phyllodytes luteolus	Brazil: Espirito Santo	N/A	12*S* and 16*S* rDNA	AY843721
Phyllodytes sp.	Brazil: Bahia	MRT 6144	12*S* and 16*S* rDNA	AY843722
Phytotriades auratus comb. nov.	Trinidad, Cerro del Aripo	T511 (N/A)	12*S* rDNA (III)	DQ403728
P. auratus comb. nov.	Trinidad, Cerro del Aripo	T511 (N/A)	16*S* rDNA	DQ403731
P. auratus comb. nov.	Trinidad, Cerro del Aripo	T511 (N/A)	cyt *b*	DQ403733
P. auratus comb. nov.	Trinidad, El Tucuche	T521 (N/A)	12*S* rDNA (I, II)	DQ403726
P. auratus comb. nov.	Trinidad, El Tucuche	T521 (N/A)	12*S* rDNA (III)	DQ403727
P. auratus comb. nov.	Trinidad, El Tucuche	T521 (N/A)	16*S* rDNA	DQ403730
P. auratus comb. nov.	Trinidad, El Tucuche	T521 (N/A)	cyt *b*	DQ403734
P. auratus comb. nov.	Trinidad, El Tucuche	T522 (N/A)	cyt *b*	DQ403735
P. auratus comb. nov.	Trinidad, El Tucuche	T523 (N/A)	cyt *b*	DQ403736
P. auratus comb. nov.	Trinidad, El Tucuche	T524 (N/A)	cyt *b*	DQ403737
P. auratus comb. nov.	Trinidad, El Tucuche	T525 (N/A)	cyt *b*	DQ403738
P. auratus comb. nov.	Trinidad, El Tucuche	T550 (N/A)	cyt *b*	DQ403739
P. auratus comb. nov.	Trinidad, El Tucuche	T551 (N/A)	cyt *b*	DQ403740

Note: Taxonomy follows Faivovich et al. (2005). AMNH-A, American Museum of Natural History (New York); ENS, Collection Eric N. Smith (Texas); CFBH, Collection Célio F. B. Haddad, Universidad Estadual Paulista (São Paulo); MVZ, Museum of Vertebrate Zoology, University of California (California); MNHNP, Museum National d'Histoire Naturelle (Paris); VSNM, National Museum of Natural History, Smithsonian Institution (Washington, DC); MACN, Museo Argentino de Ciencias Naturales (Buenos Aires); LSUMZH, Tissue Collection, Louisiana State University, Museum of Zoology (Louisiana); MRT, field numbers of Miguel Trefaut Rodrigues (São Paulo); Rds, field numbers Rafael de Sá (Virginia); UMF, University of Miami (Miami); N/A, non-available voucher.

this taxon resulted in no significant heterogeneity between partitions ($P=0.28$). The scatter-plot of uncorrected transition and transversion distances (Figure 2) was best fitted by a linear regression that shows no saturation.

Reconstruction of the relationships of *Phyllodytes* spp. to other genera within the Hylinae requires outgroup polarization. Five genera from the subfamily Hylini, sister clade to the Lophiohylini (Faivovich et al. 2005), were chosen for this purpose, and a ML tree reconstruction is shown in Figure 3. In this tree, *Phyllodytes* is paraphyletic. As in the study of Faivovich et al. (2005), *P. luteolus*+*P.* sp. are a sister clade (posterior probability PB: 1.00) to the remaining Lophiohylini, and *Osteopilus* (PB: 0.99), *Osteocephalus* (PB: 0.99) and *Trachycephalus* (PB: 1.00) form three well-supported monophyletic groups. Thus, although the phylogenetic signal may

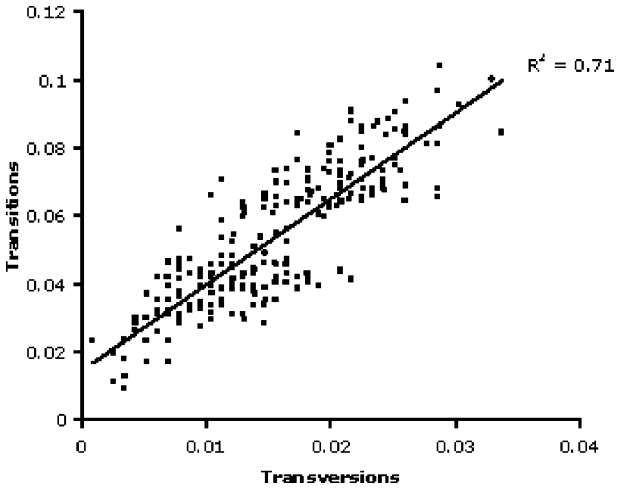

Figure 2. Scatter-plot of pairwise uncorrected transition and transversion distances estimates of the 12*S* and 16*S* rDNA genes (r^2=0.71). Outgroup taxa are excluded.

not be strong enough to resolve inter-generic relationships, it is adequate to resolve intra-generic relationships. Figure 3 shows noticeable branch-length differences between lineages and this is confirmed by relative rate tests in which most lineages showed significant differences in evolutionary rates ($P<0.05$).

Maximum likelihood pairwise distances within genera of the Lophiohylini indicate that the highest genetic distance within the ingroup is between *Phyllodytes auratus* and *P. luteolus* (18.3%) and *Phyllodytes* sp. (22.6%). Thus, divergence within *Phyllodytes* is higher than other inter-generic distances within the Lophiohylini.

Intra-population analysis reveals that with the exception of one *P. auratus* individual (T524, from El Tucuche), with one purine (A ↔ G) transition occurring in the first codon position, all remaining individuals shared the same cyt *b* haplotype. The individual from Cerro del Aripo (T511) was identical to the other six specimens from El Tucuche.

Our data show that the genus *Phyllodytes* is paraphyletic, showing approximately as much divergence as exists between other genera of the Lophiohylini. Separate generic status for *P. auratus* can therefore be justified by molecular divergence and on differences in ecology, morphology and life-history. For example, the possible lack of voice or ultrasonic communication in *P. auratus* suggests

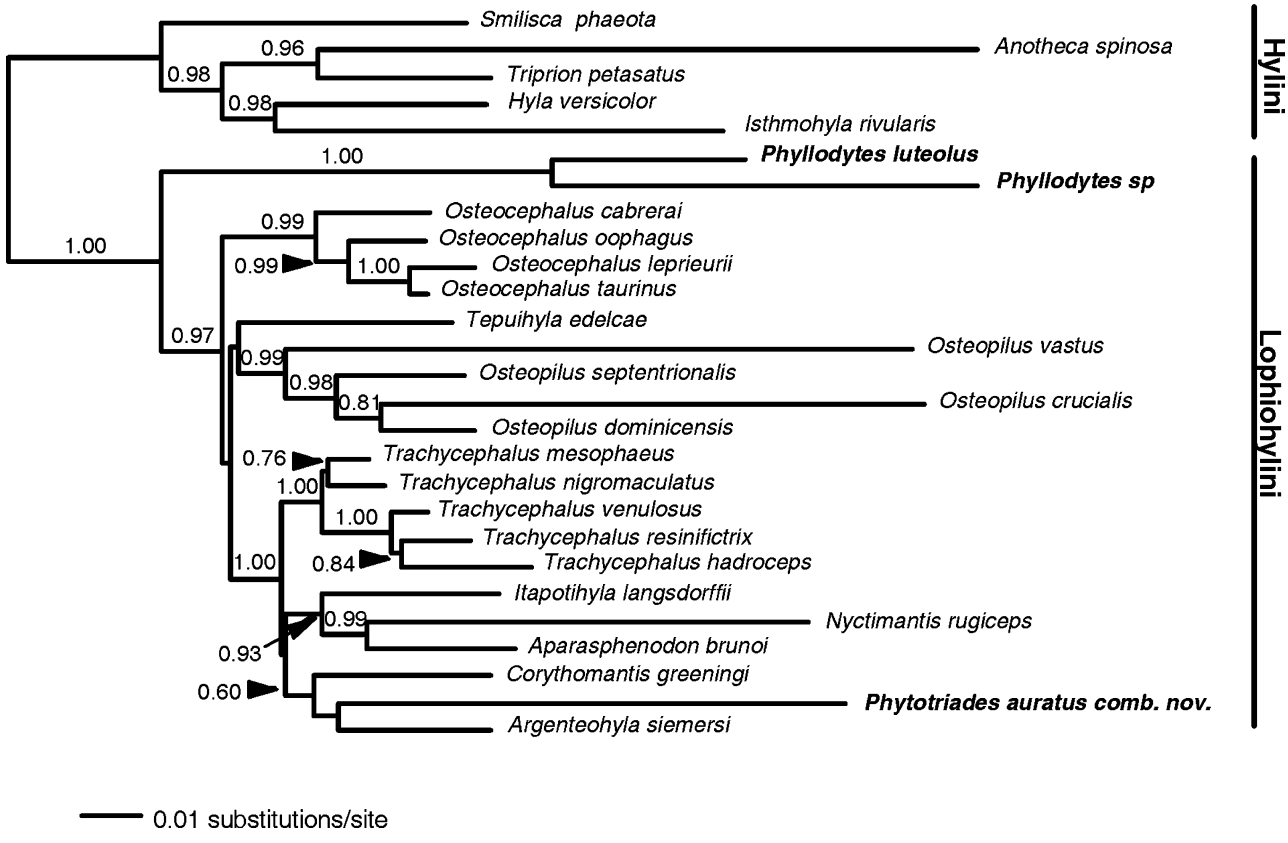

Figure 3. Maximum likelihood phylogram (−lnL=6722.3158) under a GTR+I+G model of evolution. Numbers above branches indicate posterior probabilities recovered from the Bayesian analysis (of the last 5000 trees). *Phyllodytes* spp. and *Phytotriades auratus* comb. nov. are labeled in bold type.

important mating and reproductive differences from all other *Phyllodytes* species. Recently, a study on the concave-eared torrent frog (*Amolops tormotus*) has shown that this species' advertisement call is emitted at an ultrasonic frequency, evolved to counteract the background noise of the torrents they inhabit (Feng et al. 2005). Although this aspect is beyond the scope of our study, montane bromeliad frogs might be subject to background noise during downpours and may use similar high-frequency communication. Striking differences in skin color (two iridescent yellow stripes), and restriction to only one species of cloud forest bromeliad (*G. erectiflora*) differentiates *P. auratus* from all other *Phyllodytes*. The genus *Phyllodytes* is classified mainly on the basis of odontoids and other weak morphological characters, with no clear characters unique to the genus. However, anuran fangs have evolved independently through parallelism and convergence, and large fangs are thought to have arisen as a consequence of sexual selection in several families (Fabrezi & Emerson 2003), suggesting that this character is not a reliable synapomorphy. The use of bromeliads for breeding and tadpole development has also evolved independently (e.g. Dendrobatidae and Hylidae) and is clearly not unique to the Hylinae. Thus, the monophyly of *Phyllodytes* is doubtful. Because the description of *P. luteolus* (Wied, 1824) predates that of *P. auratus* (Boulenger, 1917) and because no other name is available, we consider all species previously included in *Phyllodytes*, except the species originally described as *Amphodus auratus*, as *Phyllodytes* sensu stricto (with the following junior synonyms: *Amphodus* Peters, 1873 "1872"; *Lophyohyla* Miranda-Ribeiro, 1923) and propose a new generic name for the species originally described as *Amphodus auratus*.

Systematics

Phytotriades gen. nov.

Type species: *Amphodus auratus* Boulenger, 1917.

Diagnosis

The morphological description follows Boulenger (1917): head much depressed, a little broader than long; snout truncate, as long as the orbit, with distinct canthus and nearly vertical loreal region; nostril near the tip of the snout; interorbital space broader than the upper eyelid; tympanum hidden; a strong ridge above the temple. Fingers and toes moderately long, the tips dilated into well-developed disks. The subarticular tubercules very feeble; fingers free, first shorter than second; toes slightly webbed at the base. The tibio-tarsal articulation reaches the eye; tibia half the length of the head and body, longer than the foot. Skin smooth, coarsely granular on the belly and under the thighs. Brown above, with three golden-yellow longitudinal streaks on the back, the outer bifurcating on the head, the branches ending between and behind the upper eyelids; or head yellow, with brown spots and three brown streaks, the outer following the canthus rostralis and the supratemporal ridge. Large widely spaced vomerine-looking teeth in the lower jaw, decreasing in size from the symphysis, and small serrated teeth on the parasphenoid bone.

These morphological characters distinguish *Phytotriades* gen. nov. to all other hylids except *Phyllodytes* (all species share the presence of vomerine teeth and different species share one or more of the morphological characters described). *Phytotriades auratus* gen. nov. is distinguished from all *Phyllodytes* species by the presence of two golden longitudinal stripes on its dorsum, lack of vocalization and molecular data (mitochondrial 12*S* and 16*S* rDNA partial sequences, Appendix 1).

Distribution

The new genus is known in habitats above 600 m on the summits of El Tucuche and Cerro del Aripo (Northern Range of Trinidad, Republic of Trinidad and Tobago).

Species included

Amphodus auratus Boulenger, 1917=*Phytotriades auratus* (Boulenger, 1917) comb. nov.

Remarks

Phytotriades is strictly associated with the giant bromeliad *Glomeropitcairnia erectiflora*, two to five eggs are laid in the deposited rain water and hatched tadpoles develop by feeding on algae and infusora growing on the surface of the leaves until metamorphosis; males display territorial behavior and combat by biting with their "fangs".

Intra-population differentiation

Lack of genetic difference between the two *P. auratus* populations could indicate that El Tucuche and Cerro del Aripo were recently connected by cloud forest vegetation possibly during cooler Pleistocene conditions that would have favored the dispersal of *G. erectiflora* to colonize lower altitude localities. Thus, the *G. erectiflora* and *P. auratus* populations at

these two localities could be remnants of a once larger but now fragmented population.

Etymology

The new genus' name is derived from "Phyto" (Greek meaning plant) and "triades" (Greek for trinity, making reference to the country to which the frog is endemic).

Conservation

Our results confirm the continued presence of the Golden Tree Frog and the bromeliad *Glomeropitcairnia erectiflora* on El Tucuche and Cerro del Aripo, and their absence from Morne Bleu (Clarke et al. 1995). Although it was not an objective of our study to assess the Golden Tree Frog's population size, our results from El Tucuche are similar to those of Clarke et al. (1995) (not significantly different: $\chi^2=1.96$; $P>0.05$) but different from those at Cerro del Aripo. If our data are representative, they suggest a serious decline of this population.

Factors underlying the possible decline at Cerro del Aripo are unknown. Although human disturbance could be a factor, this seems unlikely since access to Cerro del Aripo is much the more difficult of the two peaks. A possible explanation for *P. auratus* population decline may be linked to the chytrid fungus *Batrachochytrium*, known to be most infectious at altitudes above 1000 m (e.g. La Marca et al. 2005), where the high humidity, cloud cover, and diurnal and nocturnal temperatures are at the pathogen's optimum growth range (Pounds et al. 2005; Puschendorf et al. 2006).

Not more than 206 ha of montane rain forest remain around the summits of El Tucuche and Cerro del Aripo (Clarke et al. 1995) and preservation of these rain forest areas seems to be the only method for the frog's conservation since its ecological specialization to one species of bromeliad makes a captive breeding program unlikely to succeed. Nevertheless, further study of the ecology and life history of both the Trinidad Golden Tree Frog and its host plant are desirable.

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Appendix 1. *Phytotriades auratus* comb. nov. mitochondrial sequences

Underlined bases were excluded from analysis. Numbering corresponds to *Xenopus laevis*.

12S rDNA I and II domain partial sequence

2217- cccaaccttataatcaattattacttaacatacacatgcaagtctccacactcctgtgaaaacgccttaaa
tcccttttatggataaggagctggtatcaggcacaactttagcccaaaacacctagctacgccacacccac
acgggaattcagcagtgattaacattgagcataagcgacagcttgactcagttagagtaaaaagagccggca
aatctggtgccagccgccgcggttacaccacgtggctcaaattaattaatttcggcgtaaagcgtggttaaa
attaaaaaacattatagttaaactaaaactaagttgtaacacacttgtatttaggaaaaccaaaaacgaaag
ttactataactccaacaacttgaatccacgacagttaagacccaaactggga

12S rDNA III domain partial sequence

2553- actacaagcaaagcttcaaacccaaaggacttgacggtactccatatccacctagaggagcctgtcctataa
tcgataatccccgcttaacctcaccatttttagcctcccagtctgtatacctccgtcgtcagcttacctcgt
gagcgactcttagtaagcttaatgccttacgccaacacgtcaggtcaaggtgcagcaaatgagatgggaag
agatgggctacactctctatttagaaaatacgaaaaactacctatgaaacctagtcagaaggcgaatttag
cagtaaaggggacaaaagagccccctttaatatggcactgaagtgtgt

16S rDNA partial sequence

4052- taacgtgcgaaggtagcgtaatcacttgttctttaaatgaggacttgtatgaagtggcaccacgaaagtta
cactgtctcctttttctaatcagttaaactaatctccccgtgaagaagcgaggatacaaatataagacgaga
agaccctatggagctttaaactaatataacaaatactaactcaacccaaactttaagaacaacaacctttat
tccagcattatgattattagttttaggttggggcgaccgcggagtaaaaattaacctccacattgaatgggg
actatccctgagcaaaaaccaacaacttcaagcaccaatagattgacatcaattgacccaatattttgatc
aacgaaccaagttaccctagggataacagcgcaatccatttcaagagtcctttatcgacaaatgggtttacga
cctcgatgttggatcagggtatcccagtggtgcagccgctactaaaggttcgtttgttcaacgattaaaacc
ct

Cyt b partial sequence

16675- agcgacacagttattacaaatttactttcagctgccccttacatcggcactgacctggttcaatggrtctga
ggcggattctctgtcgacaatgcaactctcacccgatttttttacatttcactttatccttccatttcttatt
gcaggagcatcaatgattcatcttcttttttcttcaccaaacaggctcatccaaccccacaggacttaactca
aacccagacaaaattccatttcattcctattattcttacaaagatattttcggcttcgcaattttacttgcc
ttcctcgcaactttatccaccttcaccccaaacatccttggagacccagataactttacaccagctaaccca
ttagtcacccctccccatattaaaccagaa