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Recommended Citation

Pinheiro, P. D. P., Kok, P. J. R., Noonan, B. P., Means, D. B., Haddad, C. F. B., & Faivovich, J. (2019). A new genus of Cophomantini, with comments on the taxonomic status of *Boana liliae* (Anura: Hylidae). *Zoological Journal of the Linnean Society*, 185(1), 226–245. <https://doi.org/10.1093/zoolinlean/zly030>

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A new genus of Cophomantini, with comments on the taxonomic status of *Boana liliae* (Anura: Hylidae)

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Received 19 December 2017; revised 27 March 2018; accepted for publication 3 April 2018

The non-monophyly of both the genus *Myersiophyla* and the *Boana punctata* group has been recovered in a number of published phylogenetic analyses. In this paper we report on the analysis of sequences of *Boana liliae*, a species originally assigned to the *B. punctata* group, in a dataset of Cophomantini that recovered novel phylogenetic relationships for this hylid tribe. Our results reveal *Myersiophyla* to be paraphyletic with respect to *B. liliae*. Support for the placement of *Myersiophyla kanaima* is poor, but this taxon is recovered as the sister taxon of the other Cophomantini genera (excluding *Myersiophyla*) or as the sister taxon of the remaining species of *Myersiophyla* (including *B. liliae*). These results lead us to propose two taxonomic changes in order to remedy the paraphyly of *Myersiophyla*: (1) a new genus is described for *M. kanaima*, and (2) *Boana liliae* is transferred to *Myersiophyla*. We further provide notes on the natural history and vocalizations of the new monotypic genus, a new diagnosis of the former *B. liliae* in the context of *Myersiophyla*, and discuss the evolution of tadpole morphology and biogeography of the earlier diverging clades of Cophomantini.

ADDITIONAL KEYWORDS: Cophomantini – Hylidae – *Myersiophyla* – systematics.

INTRODUCTION

The tribe Cophomantini was recognized by Faivovich *et al.* (2005) to include five genera of Neotropical hylids: *Aplastodiscus* Lutz, 1950, *Boana* Gray, 1825 (as *Hypsiboas* Wagler, 1830), *Bokermannohyla* Faivovich, Haddad, Garcia, Frost, Campbell & Wheeler, 2005, *Hyloscirtus* Peters, 1882 and *Myersiophyla* Faivovich, Haddad, Garcia, Frost, Campbell & Wheeler, 2005. Subsequently, different studies that focused on the

phylogenetic relationships of some of these genera increased the taxonomic sampling, particularly of *Aplastodiscus* (Berneck *et al.*, 2016), *Hyloscirtus* (Coloma *et al.*, 2012; Almendáriz *et al.*, 2014; Guayasamin *et al.*, 2015) and, to a lesser extent, *Boana* (Antunes, Faivovich & Haddad, 2008; Köhler *et al.*, 2010; Lehr, Faivovich & Jungfer, 2010; Caminer & Ron, 2014; Fouquet *et al.*, 2016; Orrico *et al.*, 2017) and *Myersiophyla* (Faivovich, McDiarmid & Myers, 2013). Higher level phylogenetic analyses (Wiens *et al.*, 2010; Pyron & Wiens, 2011; Pyron, 2014; Duellman, Marion & Hedges, 2016) obtained results congruent with those of Faivovich *et al.* (2005) or differing only in the position of poorly supported groups.

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[Version of Record, published online 6 July 2018; <http://zoobank.org/urn:lsid:zoobank.org:pub:F9F8DD5E-58DA-4F1D-AEE6-A09D49DF4972>]

Faivovich *et al.* (2005) erected the genus *Myersiohyla* for the two species of the former *Hyla aromatica* group (Ayarzagüena & Señaris, 1994), *Hyla kanaima* Goin & Woodley, 1969 (a species formerly included in the *Hyla geographica* group; Duellman & Hoogmoed, 1992) and, tentatively, *Hyla loveridgei* Rivero, 1961. Two additional species were later described and assigned to this group: *M. chamaeleo* Faivovich, McDiarmid & Myers, 2013 and *M. neblinaria* Faivovich, McDiarmid & Myers, 2013. Faivovich *et al.* (2013) also provided comments on several aspects related to the genus, and a molecular phylogenetic analysis including the two new species and *M. kanaima*. Their analysis recovered a monophyletic *Myersiohyla* with 91% jackknife support, unlike previous analyses where this was only weakly supported or not recovered (Wiens *et al.*, 2006, 2010). A subsequent re-analysis of hylid sequences in GenBank, including only sequences of *M. kanaima* and *M. neblinaria*, did not recover *Myersiohyla* as monophyletic (Duellman *et al.*, 2016), as *M. kanaima* was recovered as the sister taxon of a weakly supported clade, including *M. neblinaria* plus all other Cophomantini.

Besides supporting the monophyly of *Myersiohyla*, the results of Faivovich *et al.* (2013) also indicated the non-monophyly of the *Boana punctata* group as tentatively defined by Faivovich *et al.* (2005), due to both the position of *B. ornatissima* (Noble, 1923), which was recovered nested in the *B. benitezi* group, and the poorly supported position of *B. sibleszi* (Rivero, 1972), recovered as the sister taxon of the *B. semilineata* group. Considering that the monophyly of the remaining taxa, i.e. *B. cinerascens* (Spix, 1824), *B. picturata* (Boulenger, 1899) and *B. punctata* (Schneider, 1799), was only weakly supported, and that some species assigned to the group—*B. alemani* (Rivero, 1964), *B. atlantica* (Caramaschi & Velosa, 1996), *B. hobbsi* (Cochran & Goin, 1970), *B. jimenezi* (Señaris & Ayarzagüena, 2006) and *B. liliae* (Kok, 2006)—were not available for the analysis, Faivovich *et al.* (2013) were explicit about the need for a stringent test of the monophyly of this species group. The absence of phenotypic synapomorphies for several species groups of *Boana* and the absence of a prepollical spine in *B. liliae* made its assignment to the *B. punctata* group tentative, based on its overall similarity with *B. cinerascens* (Kok, 2006).

In this paper we report the results of including samples of *B. liliae* in a phylogenetic hypothesis that proved relevant for testing the monophyly of both the *B. punctata* group and *Myersiohyla*, and the recognition of a new genus of Cophomantini. We further provide notes on the natural history and vocalizations of the new monotypic genus, a new diagnosis for *Boana liliae* based on its new taxonomic context and a brief discussion of larval character evolution and biogeography of the earlier diverging clades of Cophomantini based on our phylogenetic results.

MATERIAL AND METHODS

TAXON SAMPLING

Our dataset included the same taxonomic sampling employed by Faivovich *et al.* (2013), complemented with additional species of Cophomantini subsequently sequenced by Caminer & Ron (2014), Guayasamin *et al.* (2015), Fouquet *et al.* (2016), Berneck *et al.* (2016) and Orrico *et al.* (2017), and new sequences from four specimens of *B. liliae* [IRSNB (Institut Royal des Sciences Naturelles de Belgique, Brussels, Belgium) 1965 and 1968, holotype and paratype, respectively, from Kaieteur Plateau, Potaro-Siparuni District, Guyana; UTA-A (University of Texas, Arlington, USA) 63335 and 63336 from Imbaimadai, Cuyuni-Mazaruni District, Guyana], and two specimens of *M. kanaima* (IRSNB 16751 and 16753, from Wokomung Massif, Potaro-Siparuni District, Guyana), for a total of 129 terminals. See Supporting Information S1 for GenBank accession numbers. The recognition of tribes in the subfamily Hylinae follows the recent discussion of hylid taxonomy of Faivovich *et al.* (2018).

CHARACTER SAMPLING

We included up to 7486 base pairs (bp) per terminal from the same gene fragments employed by Faivovich *et al.* (2013): cytochrome *b* (*Cytb*), 12S, tRNA^{VAL}, and 16S (*H1*), tRNA^{LEU}, NADH dehydrogenase subunit 1 (*ND1*), and tRNA^{ILE} mitochondrial genes, and also fragments of seven in absentia homologue 1 (*SIA*), exon 1 of rhodopsin (*RHOD*), tyrosinase (*TYR*), recombination activating gene 1 (*RAG1*), exon 2 of chemokine receptor 4 (*CXCR4*), and 28S nuclear genes. The primers are those used by Faivovich *et al.* (2013).

DNA ISOLATION AND SEQUENCING

The DNA isolation, polymerase chain reaction (PCR) and sequencing protocols are those described by Faivovich *et al.* (2013). Sequences were aligned using MAFFT (Katoh & Toh, 2008). For the protein coding genes (i.e. *Cytb*, *ND1*, *SIA*, *RHOD*, *TYR*, *RAG1* and *CXCR4*) we used the G-INS-I strategy, and for the non-coding genes *H1* and 28S we used AUTO-FFT-NS-2. All other parameters were set as default. Alignments were edited using BioEdit (Hall, 1999) and sequence files were merged with SequenceMatrix (Vaidya, Lohman & Meier, 2011).

PHYLOGENETIC ANALYSIS

Phylogenetic analyses were conducted using parsimony as the optimality criterion. The rationale for this is explained by Farris (1983) and discussed by others, such as Goloboff (2003) and Goloboff & Pol (2005). The searches were done with TNT Willi Hennig Society

Edition (Goloboff, Farris & Nixon, 2008), using New Technology Searches, combining Sectorial Search, Parsimony Ratchet, Tree Drift, Tree Fusing and requesting the driven search to hit the best length 100 times. Analyses were run alternatively with gaps treated as a fifth state or as missing data. Parsimony jackknife absolute frequencies (Farris *et al.*, 1996) were calculated with the same parameters as the searches for the best score, although requesting the driven search to hit the best length ten times, for a total of 1000 replicates.

We also performed a Bayesian analysis using MrBayes v.3.2 (Ronquist *et al.*, 2012). Models for each partition were selected with PartitionFinder v.2.1.1 (Lanfear *et al.*, 2016). Branch lengths were treated as linked and the analysis considered only models employed by MrBayes v.3.2. The corrected Akaike Information Criterion (AICc) was used to select the best-fitting model for each gene (Lanfear *et al.*, 2016). First, second and third codon positions were treated as separate partitions for each protein-coding gene. Sequence fragments of 12S, tRNA^{VAL}, 16S, tRNA^{LEU}, and tRNA^{ILE} were treated as a single partition for model selection. PartitionFinder was run employing the greedy algorithm (Lanfear *et al.*, 2012) and PhyML software (Guindon *et al.*, 2010). Bayesian analysis was performed in the CIPRES web cluster (Miller, Pfeiffer & Schwartz, 2010). Two runs of 60 million generations with four Monte-Carlo Markov Chains each (with a burn-in fraction of 0.25), state frequencies (statefreqpr) set as fixed (equal) and substitution rate (ratepr) was set to variable. The other priors were set as default. Stabilization of resulting parameter estimates was evaluated using Tracer (Rambaut *et al.*, 2014). Bayesian results with the nodes Posterior Probabilities (PP) are presented as Supporting Information S2.

Uncorrected *p*-distances were estimated by pairwise comparisons of the final ~500 bp sequences of the 16S mitochondrial rRNA gene (see Vences *et al.*, 2005a, b; Fouquet *et al.*, 2007) using PAUP* (Swofford, 2002). Trees were edited with FigTree (Rambaut, 2014).

CALL DESCRIPTION

One unvouchered specimen of *Myersiophyla kanaima* was recorded at the Wokomung Massif, Potaro-Siparuni district, Guyana. Recording was made using a Zoom H4N Handy Recorder coupled to a Rode NTG-2 directional shotgun condenser microphone, 1.5 m distant from the frog. The data were analysed using software Raven Pro 64 v.1.4 (Cornell Lab of Ornithology, Ithaca, NY). Spectrograms and power spectra were produced with a window size of 256 samples, 75% overlap, hop size of 64 samples and window type Hamming. Resolution, contrast and brightness were those of the default settings. The recording is housed at Coleção Bioacústica do Centro de Coleções Taxonômicas

da Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, under the number CBUFMG 990.

The following acoustic parameters were considered: number of notes, note duration (time from the beginning to the end of one note, measured on the oscillogram), interval between notes (time from the end of one note to the beginning of the following note, measured on the oscillogram), dominant frequency range (band of frequency in which the energy of the note is concentrated, measured on the spectrogram) and peak frequency (the specific frequency with higher energy of the note, provided by Raven Pro). The terminology for call description is that of Köhler *et al.* (2017).

RESULTS

PHYLOGENETIC ANALYSES

From the total of 7486 aligned characters, 2828 were informative (~38%). The static parsimony phylogenetic analyses (gaps treated as a fifth state) resulted in eight trees of 28 819 steps (Figs 1, 2). The conflict between these optimal hypotheses involves internal relationships of the *Aplastodiscus albofrenatus* group, the *Boana faber* and *B. pulchella* groups, and among specimens of *Myersiophyla neblinaria*. The few topological differences between parsimony results (considering gaps as a fifth state or as missing data) and Bayesian hypotheses involve clades with jackknife values below 70% in the parsimony analyses, including relationships among outgroups, the position of *M. kanaima*, internal relationships of a few taxa in *Aplastodiscus*, *Bokermannohyla*, and *Hyloscirtus*, the relationships of the *Boana benitezi*, *B. punctata* and *B. semilineata* groups, *B. picturata* and *B. sibleszi* with respect to other species groups of *Boana*, and the internal relationships of the *B. albopunctata* group.

The four specimens of *Boana liliae* are recovered nested within *Myersiophyla*, as the sister taxon of *M. chamaeleo* (97% jackknife with gaps treated as a fifth state; 99% when treated as missing data), rendering *Myersiophyla* paraphyletic (Fig. 1). When we exclude *Boana liliae*, we obtain a monophyletic *Myersiophyla* with 86% jackknife support (gaps as fifth state) or 77% support (gaps as missing data). Considering gaps as a fifth state, the inclusion of *B. liliae* diminishes the support for the clade including *M. kanaima*, *M. neblinaria*, *M. chamaeleo* and *B. liliae* (<50%). When gaps are treated as missing data, *M. kanaima* is recovered as the sister taxon of the clade including *Hyloscirtus*, *Bokermannohyla*, *Aplastodiscus* and *Boana*, with <50% jackknife support (Fig. 3); a similar position is recovered in the Bayesian analysis (PP 0.998; Supporting Information S2).

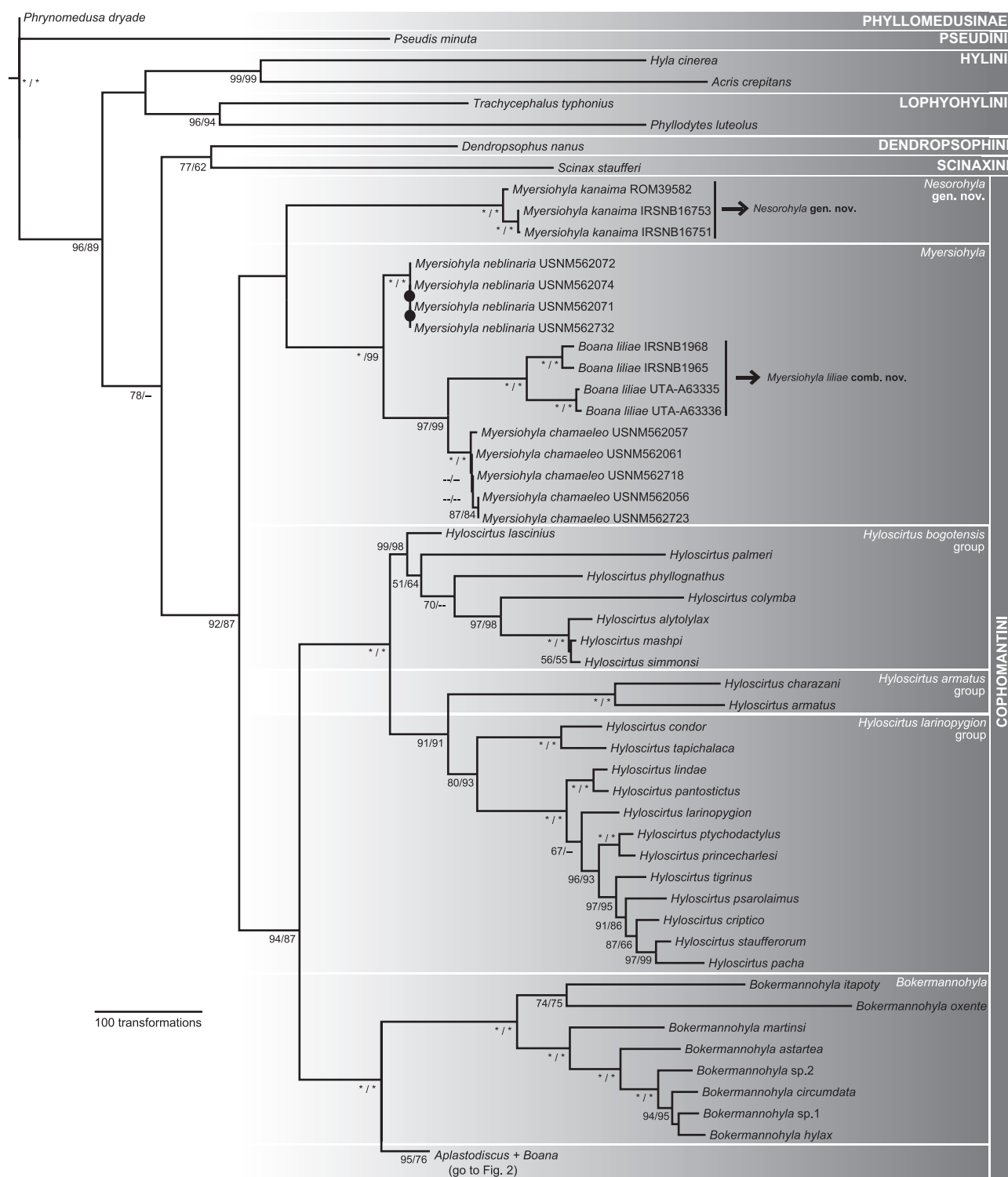
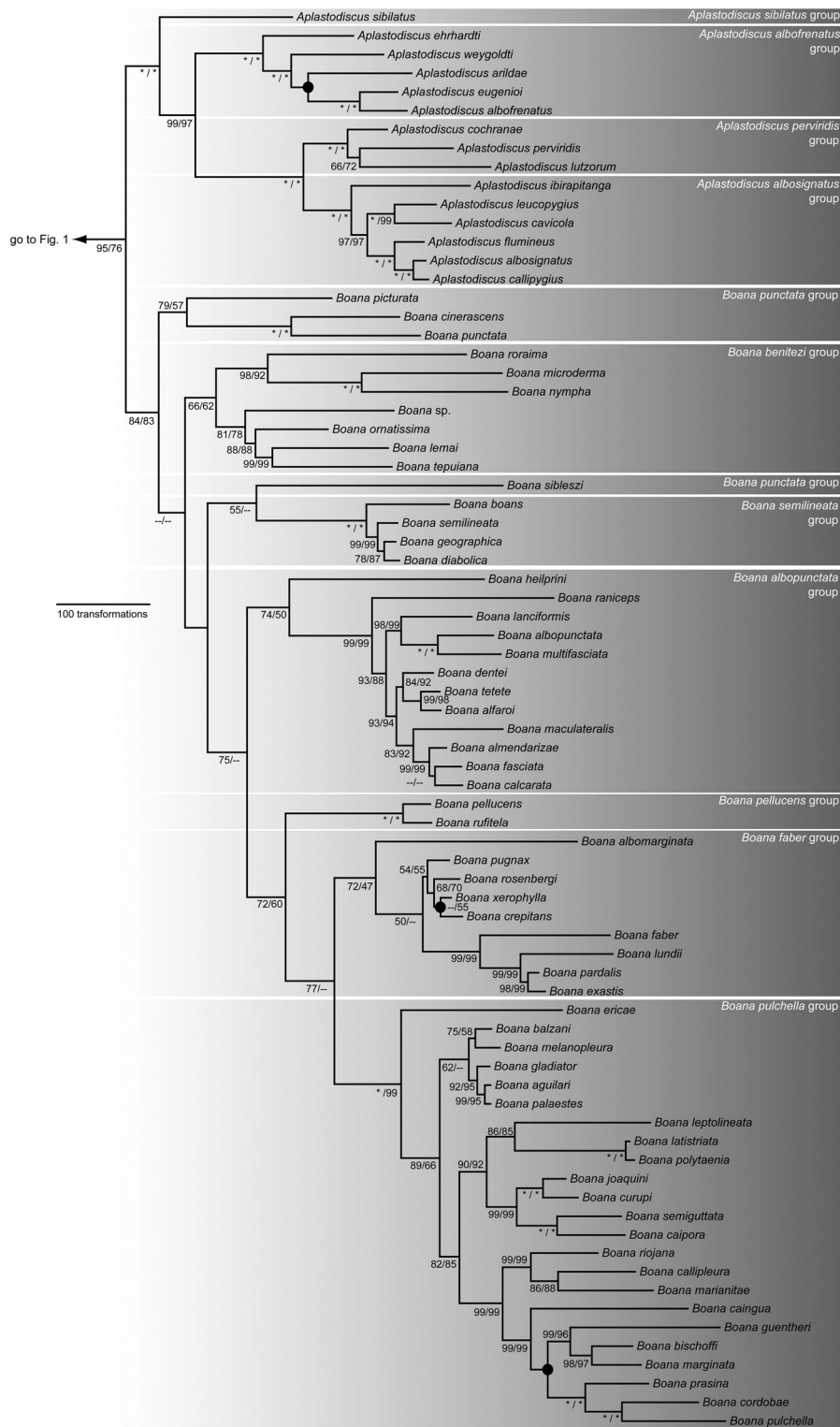


Figure 1. Partial view of one of the eight most parsimonious trees of 28 819 steps from a parsimony analysis in TNT treating gaps as a fifth state. Black dots indicate nodes that collapse in the strict consensus. Numbers on nodes are Parsimony jackknife values for analyses treating gaps as a fifth state / missing data. Nodes without values were not recovered in the analysis treating gaps as missing data. An asterisk indicates 100% jackknife value; a double dash (--) a value <50%.



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Figure 2. Partial view of one of the eight most parsimonious trees of 28 819 steps from a parsimony analysis in TNT treating gaps as a fifth state (continued from Fig. 1). Black dots indicate nodes that collapse in the strict consensus. Numbers on nodes are Parsimony jackknife values for analyses treating gaps as a fifth state/missing data. Nodes without values were not recovered in the analysis treating gaps as missing data. An asterisk indicates 100% jackknife value; a double dash (--) a value <50%.

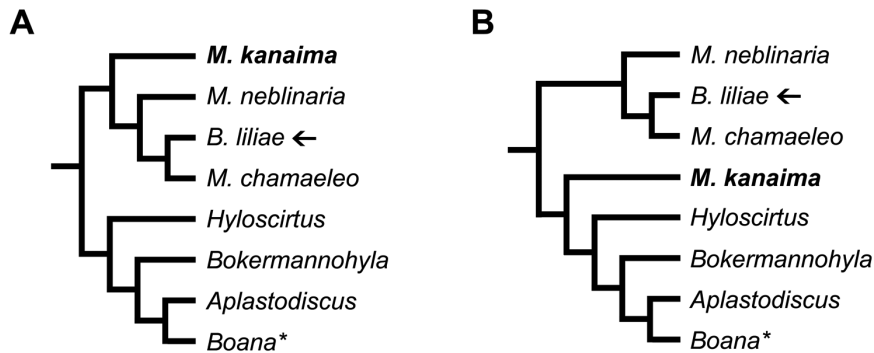


Figure 3. Pruned trees showing the alternative position of *Myersiophyla kanaima* (in bold) in the tribe Cophomantini in the different analyses. A, parsimony analysis treating gaps as fifth state. B, parsimony analysis treating gaps as missing data, and Bayesian analysis (see Supporting Information 2). An asterisk in *Boana* indicates its non-monophyly, as in all analyses *Boana liliae* (black arrow) is nested in *Myersiophyla*.

The *Boana punctata* group is recovered as non-monophyletic due to the position of *B. liliae* nested within *Myersiophyla*. Also, *B. sibleszi* is recovered with poor support (55% jackknife support with gaps treated as a fifth state; <50% when treated as missing data) as the sister taxa of the *B. semilineata* group (Fig. 2). *Boana picturata*, *B. punctata* and *B. cinerascens* are recovered as monophyletic, with 75% jackknife support when treating gaps as a fifth state and 57% when treating them as missing data. In the Bayesian analysis, *B. picturata* is recovered with weak support (PP 0.776) as the sister taxon of the clade including *B. albopunctata*, *B. pellucens*, *B. faber* and *B. pulchella* groups. *Boana sibleszi* is recovered, also with weak support (PP 0.667), as the sister taxon of the *B. benitezi* group. *Boana cinerascens* and *B. punctata* are recovered as the sister taxon of the *B. semilineata* group (PP 0.603; Supporting Information S2).

TAXONOMIC CHANGES TO PRESERVE THE MONOPHYLY OF MYERSIOHYLA

Our findings revealed the non-monophyly of *Myersiophyla*, since *Boana liliae* is nested within it as the sister taxon of *M. chamaeleo*. *Myersiophyla kanaima* is recovered as the poorly supported (<50% jackknife) sister taxon of the remaining species of *Myersiophyla* in the parsimony analysis considering gaps as a fifth state, or as the sister taxon of the other genera of Cophomantini (*Aplastodiscus*, *Boana*, *Bokermannohyla*, and *Hyloscirtus*) in the parsimony analysis considering gaps as missing data and in the Bayesian analysis. Several other analyses (Wiens *et al.*, 2006, 2010; Pyron & Wiens, 2011; Duellman *et al.*, 2016) have reported results similar to ours with respect to the possible non-monophyly of *Myersiophyla*. However, none of the analyses published subsequently to Faivovich *et al.* (2013)—where the monophyly of

Myersiophyla was supported with 91% jackknife—included the same three species of *Myersiophyla* employed by those authors.

Based on our results, it is necessary to remedy the non-monophyly of both *Myersiophyla* and the *Boana punctata* group. The most stable solution for this is the recognition of a new genus for *M. kanaima* and the formal inclusion of *Boana liliae* in *Myersiophyla*.

A NEW GENUS OF COPHOMANTINI

NESOROHYLA GEN. NOV.

Type species: *Hyla kanaima* Goïn & Woodley, 1969.

Diagnosis: (1) Enlarged prepollex, not modified as a projecting spine; (2) reduced fringes on fingers and toes; (3) nuptial pads present, light-coloured, on inner margin of Finger II (digits numbered as Fabrezi & Alberch, 1996) and inner metacarpal tubercle; (4) two small calcar tubercles; (5) dorsal coloration overall brownish; (6) iris of adults are black under natural light conditions; (7) tadpole oral disc with short, anterior and posterior gaps on marginal papillae; (8) three emarginations on the posterior labium; (10) labial tooth row formula (LTRF) 2(2)/4[1]; (11) eggs entirely pigmented.

Included species: *Nesorohyla kanaima* (Goïn & Woodley, 1969) new combination.

Sister taxon: Obtained in alternative, weakly supported (<50% jackknife) positions as the sister taxon of *Myersiophyla* or as the sister taxon of *Hyloscirtus* + *Bokermannohyla* + *Aplastodiscus* + *Boana*.

Etymology: The name *Nesorohyla* is derived from the combination of Greek roots *nesos* (island) and *oros* (mountain), and the classical genus *Hyla*, meaning *Hyla* from the mountain island. It is an allusion to the geologic formation where the species is found: the

tepui in northern South America, which are usually referred to as altitude islands. The gender is feminine.

Characterization: The only species included in this genus was also characterized by Duellman & Hoogmoed (1992) and MacCulloch & Lathrop (2005).

Comparison with other genera of Cophomantini: From the characters employed in the diagnosis, the only putative autapomorphies of *Nesorohyla* so far seem to be the completely pigmented ovum (Duellman & Hoogmoed, 1992; Faivovich et al., 2013) and, possibly, the coloration of the iris in adults (described as black by MacCulloch & Lathrop, 2005). Field experience of some of the authors shows that under natural light conditions, the iris of adults appears to be black. This is because it is so densely coloured with melanin that it is not distinguishable from the pupil, unless one shines a bright light on it. For this reason, we refer to the iris of *Nesorohyla kanaima* as being black. The value of the generic diagnoses that are not based on synapomorphies is extremely limited, as they are of actual use only for species already known to be included in those genera—and on the basis of which the generic diagnosis is made—but have no predictive value for the inclusion of new species. We provide below a comparison of *Nesorohyla*, based on the diagnostic characters, with all other genera of Cophomantini.

The occurrence of completely black ova and black iris in adults differentiates *Nesorohyla* from all other genera of Cophomantini. The reduced fringes on fingers and toes differentiate *Nesorohyla* from all species of *Hyloscirtus* (Faivovich et al., 2005; Rivera-Correa & Faivovich, 2013). The absence of a prepollical spine differentiates *Nesorohyla* from most species in the genera *Boana* and *Bokermannohyla* (Faivovich et al., 2005). The small, light-coloured nuptial pad differentiates *Nesorohyla* from *Myersiophyla* (dark coloured simple or double nuptial pads covering the medial margin of Finger II, including Prepollex and Metacarpal II; Faivovich et al., 2013). The calcar with two small tubercles distinguishes *Nesorohyla* from *Myersiophyla* [calcar absent in *M. aromatica* (Ayarzagüena & Señaris, 1994), *M. chamaeleo*, *M. inparquesi* (Ayarzagüena & Señaris, 1994), *M. liliae*, and *M. neblinaria*; calcar as a transversal ridge on the heel in *M. loveridgei* (Rivero, 1961; Ayarzagüena & Señaris, 1994; Kok, 2006; Faivovich et al., 2013)]. The overall dorsal brownish coloration differentiates *Nesorohyla* from *Aplastodiscus* and most species in the *Hyloscirtus bogotensis* group (overall green, with only one brown species in *Aplastodiscus*; Guayasamin et al., 2015; Berneck et al., 2016).

The tadpoles of *Nesorohyla* can be distinguished from those of *Myersiophyla* by having an oral disc with short anterior and posterior gaps on marginal papillae (gaps absent in *M. aromatica*, *M. chamaeleo*, *M. inparquesi*

and *M. neblinaria*; Ayarzagüena & Señaris, 1994; Faivovich et al., 2013); three emarginations on the posterior labium: one medial, two posterolateral (emarginations absent in *M. aromatica*, *M. chamaeleo* and *M. inparquesi*; Ayarzagüena & Señaris, 1994; Faivovich et al., 2013); LTRF 2(2)/4[1] (combined LTRF 4–16/7–21 in *M. aromatica*, *M. chamaeleo*, *M. inparquesi* and *M. neblinaria*; Ayarzagüena & Señaris, 1994; Faivovich et al., 2013).

Call description: The call of *Nesorohyla kanaima* from the Wokomung Massif is normally composed of a single note ($N = 6$). However, a call of two notes was recorded once. The notes have a pulsatile structure, with 1–5 poorly resolved pulses, and their duration is 46–106 ms (80 ± 17 ms; $N = 8$). The call composed of two notes has a note interval of 141 ms. Seven calls were emitted in a 29.8 s interval (call rate of 14.08 calls/min). The dominant frequency band occurs between 1872.2 and 2755.4 Hz, with peak frequency at 2239.5 Hz ($N = 5$) or 2411.7 Hz ($N = 3$). There is no modulation in frequency during the call. From the notes with peak frequency of 2411.7 Hz, one has two more harmonics (less energetic): one at 4823.4 Hz and the other at 7235.1 Hz (Fig. 4). Sometimes an acute groan preceded or was emitted after the call, but unfortunately these notes were not of suitable quality for analysis.

Goin & Woodley (1969) reported the call of *Nesorohyla kanaima* from the type locality as composed of two notes of two to four pulses, with dominant frequency between 3400 and 3800 Hz. It is unclear if the differences between vocalizations from Mt. Kanaima and Wokomung Massif are due to interpopulation variation, recording artefacts or if there is more than one species under the name *Nesorohyla kanaima*.

Comparison with other calls of basal Cophomantini: From species of *Myersiophyla*, the call of *Nesorohyla kanaima* differs from those of *M. aromatica*, *M. inparquesi* and *M. neblinaria*, by having pulsatile structure with 1–5 poorly resolved pulses (6–11 pulses in those species combined; Ayarzagüena & Señaris, 1994; Faivovich et al., 2013). From *M. liliae*, it differs by having one to two notes (bout of 214–222 notes in *M. liliae*; Kok, 2006). From *M. chamaeleo*, it differs by having the dominant frequency coincident with the fundamental frequency (dominant frequency at the second harmonic in *M. chamaeleo*; Faivovich et al., 2013).

The call of *Nesorohyla kanaima* can be differentiated from those of *Hyloscirtus alytolylax* (Duellman, 1972), *H. colymba* (Dunn, 1931), *H. mashpi* (Guayasamin et al., 2015), *H. phyllognathus* (Melin, 1941), *H. platydactylus* (Boulenger, 1905), *H. simmonsii* (Duellman, 1989), *H. torrenticola* (Duellman & Altig, 1978), *H. criptico* Coloma, Carvajal-Endara, Dueñas,

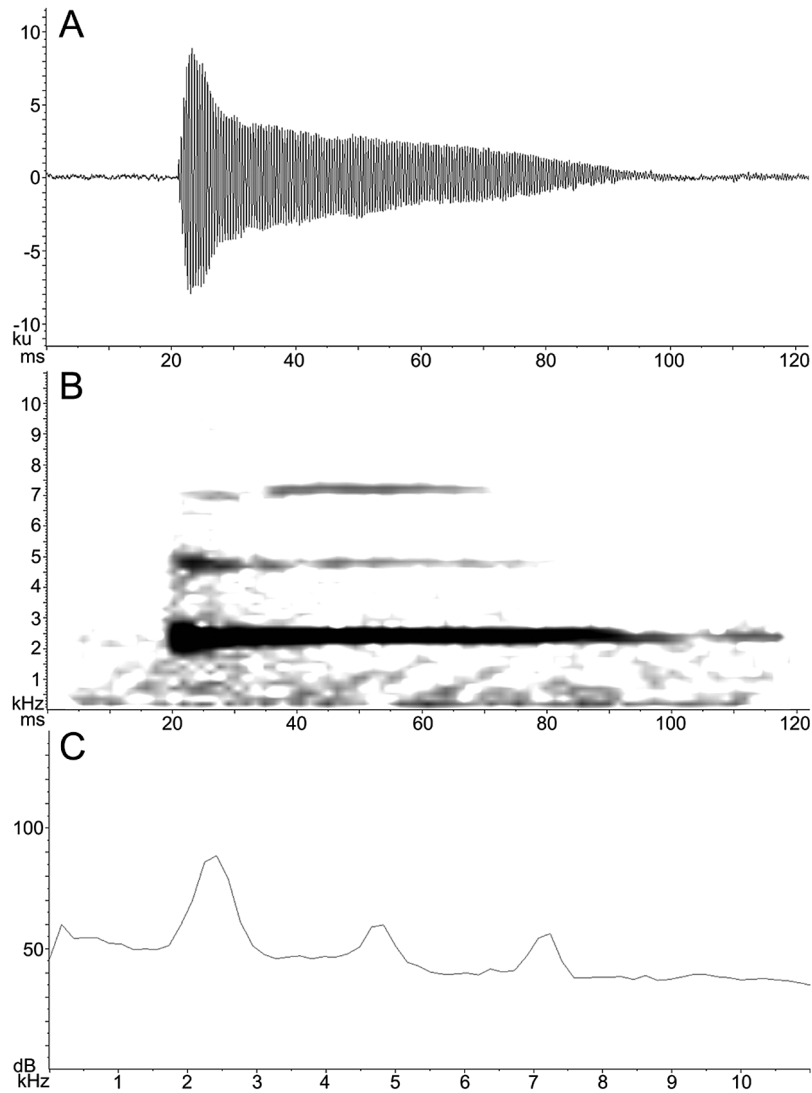


Figure 4. Advertisement call of an unvouchered specimen of *Nesorohyla kanaima* from Wokomung Massif, Potaro-Siparuni District, Guyana (5.002222°N, 59.879722°W; 1540 m). A, waveform; B, spectrogram; C, power spectrum of the call composed by one note of one pulse and three harmonic bands. Recorded on 23 June 2012, 21:30 h, air temperature 20 °C (recording CBUFMG 990).

Paredes-Recalde, Morales-Mite, Almeida-Reinoso, Tapia, Hutter, Toral-Contreras & Guayasamin, 2012, *H. pacha* (Duellman & Hillis, 1990), *H. staufferorum* (Duellman & Coloma, 1993) and *H. tapichalaca* (Kizirian, Coloma & Paredes-Recalde, 2003) by having 1–2 notes (calls emitted in bouts of 2–104 notes in those species combined; Duellman, 1972; Duellman & Altig, 1978; La Marca, 1985; Duellman & Coloma, 1993; Kizirian, Coloma & Paredes-Recalde, 2003; Coloma *et al.*, 2012; Guayasamin *et al.*, 2015). From *H. jahni* (Rivero, 1961), *H. palmeri* (Boulenger, 1908), *H. torrenticola*, *H. antioquia* (Rivera-Correa & Faivovich, 2013), *H. criptico*, *H. larinopygion* (Duellman, 1973), *H. pantostictus* (Duellman & Berger, 1982),

H. psarolaimus (Duellman & Hillis, 1990), *H. staufferorum* and *H. tapichalaca*, it differs by the lack of frequency modulation (frequencies with ascending modulation in those species; Duellman & Altig, 1978; La Marca, 1985; Duellman & Coloma, 1993; Kizirian *et al.*, 2003; Coloma *et al.*, 2012; Rivera-Correa & Faivovich, 2014; Rivera-Correa, Vargas-Salinas & Grant, 2017). From *H. armatus* (Boulenger, 1902) and *H. condor* (Almendáriz, Brito, Batallas & Ron, 2014), the call of *N. kanaima* differs by being shorter (note duration 46–106 ms in *N. kanaima*, 160–240 ms in *H. armatus*, 286–915 ms in *H. condor*; Duellman, De la Riva & Wild, 1997; Almendáriz *et al.*, 2014) and by its peak frequency (2239.5 or 2411.7 Hz in *N. kanaima*;

2500 or 4700 Hz in *H. armatus*; 770–1270 Hz in *H. condor*; Duellman *et al.*, 1997; Almendáriz *et al.*, 2014).

Natural history: Few observations have been made on the biology of *Nesorohyla kanaima*. Duellman & Hoogmoed (1992) reported the absence of ponds on the slopes of Mt. Roraima (limits between south-eastern Venezuela, Guyana and north-eastern State of Roraima, Brazil), and associated this to the large oviducal eggs of females to suggest that probably the species reproduces in rivers and rivulets. MacCulloch & Lathrop (2005) collected tadpoles and juveniles in a lentic riverine pond on Mt. Ayanganna.

On the eastern flanks of Mt. Roraima (Guyana), adults and a metamorph of *Nesorohyla kanaima* were observed on arboreal branches at heights below 3 m, close to cascading mountain streams. In the pools of those streams, 1–1.5 m deep, there were plenty of densely black-coloured tadpoles. Frogs were found between 808 (5.267361°N, 60.691389°W) and 1244 (5.259207°N, 60.720227°W) m a.s.l. in the adjacent woodland habitats. In the same habitats also were found adults of *Boana lemai* (Rivero, 1972), *B. roraima* (Duellman & Hoogmoed, 1992), *B. sibleszi*, *Oreophrynella macconnelli* Boulenger, 1900, *Otophryne robusta* Boulenger, 1900, *Pristimantis* cf. *saltissimus*, *Stefania roraimae* Duellman & Hoogmoed, 1984, *Stefania* sp., *Tepuihyla* sp. and an unidentified bufonid.

On the Wokomung Massif (Potaro-Siparuni, Guyana), *Nesorohyla kanaima* was found to be the most abundant anuran between 708 to 1540 m a.s.l. in primary cloud forests. Expeditions passing through the north-eastern (5.13°N, 59.81333°W), north-western (5.110083°N, 59.820583°W), central (5.090278°N, 59.838333°W) and south-eastern (5.002222°N, 59.879722°W) regions of the Massif were conducted during June, July and December of different years. Adults and metamorphs were found perched from low bushes to branches up to three meters high along cascading streams and in adjacent forests. During the mid-year rainy season, dense aggregations of males were found in low vegetation next to, or overhanging, slow-water pools of the streams, or along small, swampy creeks. They were in cacophony, emitting their call composed of one whistled note (see call description above). Both sexes presented noxious smelling skin secretions that tasted bitter. On one occasion, five males of *N. kanaima* were collected and kept in the same plastic bag as breeding males of *Boana lemai*, whose skin secretions were perceived to be more pungently smelling and bitter tasting. Two hours later the specimens of *N. kanaima* were dead, probably due to the skin secretions of *B. lemai* that had foamed up in

the bag. The specimens from the latter species were still alive.

The black tadpoles were found at high density in almost all, small to large, streams visited. They were not fast swimmers and they were not schooling. No fish were observed in those streams, but two species of freshwater crabs were found as potential predators: *Kunziana irengis* (Pretzmann, 1971) and *Microthelphusa meansi* Cumberlidge, 2007. Other frogs found in the same habitat of *Nesorohyla kanaima* on the Wokomung Massif were *Adenomera lutzi* Heyer, 1975, *Anomaloglossus beebei* (Noble, 1923), *A. kaiei* (Kok, Sambhu, Roopsind, Lenglet & Bourne, 2006), *Atelopus hoogmoedi* Lescure, 1974, *Boana lemai*, *B. roraima*, *B. sibleszi*, *Oreophrynella macconnelli*, *Otophryne robusta*, *O. steyermarki* (Rivero, 1968), *Pristimantis dendrobatoides* (Means & Savage, 2007), *P. marmoratus* (Boulenger, 1900), *P. saltissimus* (Means & Savage, 2007), *Stefania ayangannae* (MacCulloch & Lathrop, 2002), *S. coxi* (MacCulloch & Lathrop, 2002), *S. roraimae*, *Stefania* sp., *Tepuihyla warreni* (Duellman & Hoogmoed, 1992), *T. exophthalma* (Smith & Noonan, 2001) and an unidentified bufonid.

Distribution: *Nesorohyla kanaima* is known from the Pakaraima Mountains in the Guiana Highlands, in eastern Venezuela (Estado Bolívar) and western Guyana (Cuyuni-Mazaruni District). It has been collected in Mt. Ayanganna (MacCulloch & Lathrop, 2005), Mt. Kanaima, (Goin and Woodley, 1969), Mt. Roraima (Duellman & Hoogmoed, 1992), Wokomung and Maringma-tepui (Kok & Means, pers. obs.).

Remarks: Our study of some adult male specimens [vouchers ROM (Royal Ontario Museum, Toronto, Canada) 39 575–76, 43 861, 43 871, from Mount Ayanganna, western Guyana] did not reveal a mental gland, but this needs to be corroborated histologically. The polarity of most phenotypic diagnostic characters is unclear, with the exception of the entirely pigmented ova and possibly of the black iris in adults (but see Discussion), which are putative autapomorphies of this new genus. Observations of metamorphosing individuals (Fig. 5A–C) show that the iris is black and speckled with silver, light bronze, and copper (as described in field notes on live specimens from both PJR Kok and DB Means). The pupil is slightly heart-shaped and is delimited by a bronze pupil ring. In adults it turns black to a point where the pupil is difficult to distinguish, unless shining a bright light on it (Fig. 5D–E).

The tadpoles described by MacCulloch & Lathrop (2005) were tentatively assigned to *Nesorohyla kanaima* due to the presence of both juveniles and recently metamorphosed individuals, associated with

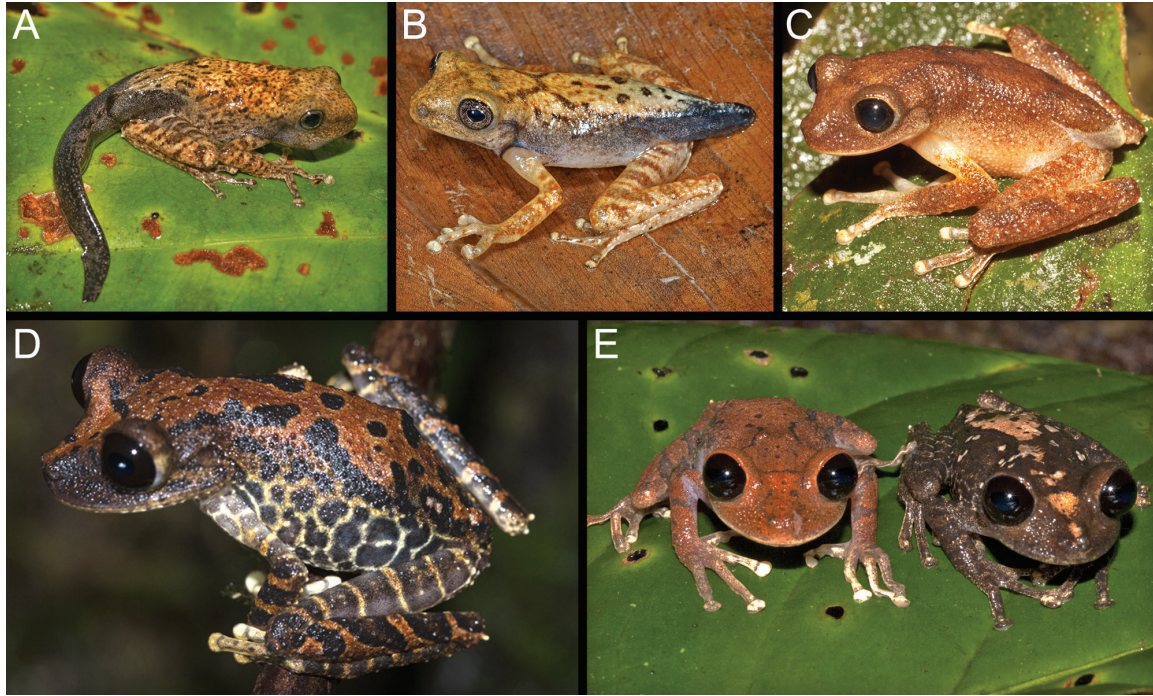


Figure 5. Ontogenetic changes in the iris coloration of *Nesorohyla kanaima*. A, B, in metamorphs the iris is speckled with silver, light bronze and copper on a black background. C, in a recently metamorphosed specimen, the motling is darker and less evident. D, E, adults have a black iris. A, D, and E are from the summit of the Wokomung Massif; B, from the Maringma-tepui; C, from south-east region of Mt. Roraima, Guyana (A, C, D, E, unvouchered specimens; B, IRSNB 16764).

the species by the colour pattern. The identification of these tadpoles was discussed by [Faivovich *et al.* \(2013\)](#), who raised the possibility of a misidentification. We obtained additional tadpoles from Wokomung Massif (IRSNB 16763) and the slopes of Maringma-tepui (IRSNB 16765) in Guyana for which identity with *Nesorohyla kanaima* has been established by molecular analyses (PJR Kok, unpubl. data). These tadpoles are similar to those described by [MacCulloch & Lathrop \(2005\)](#), leaving no doubt that these authors indeed described the tadpole of *Nesorohyla kanaima*.

The study of our tadpoles indicates that the first anterior tooth row (A1) does not have a medial gap as described by [MacCulloch & Lathrop \(2005\)](#), but form a medial acute angle that we believe could had led those authors to misinterpret it as a medial gap. The LTRF on studied specimens varied from 2(2)/4 ($N = 5$) to 2(2)/4(1) ($N = 8$). Also, [MacCulloch & Lathrop \(2005\)](#) stated that the oral disc of tadpoles from Mount Ayanganna is not emarginated. Our study of tadpoles from Maringma-tepui revealed the presence of three emarginations on the posterior labium: one medial and two posterolateral. The nostrils of studied specimens have a large, medial, fleshy projection, that when pushed against the nostril, almost closes it.

A SIXTH SPECIES OF *MYERSIOHYLA*

Our results indicate that *Boana liliae* should be transferred to *Myersiohyala*. The species is recovered as the sister taxon of *M. chamaeleo*, with 97% jackknife support (PP 1.0). We present below a new diagnosis for the species that we recognize as:

MYERSIOHYLA LILIAE (KOK, 2006) COMB. NOV.

Hypsiboas liliae Kok, 2006: 192, figs 1–4.

Hypsiboas liliae Kok — [Kok & Kalamandeen, 2008](#): 172, fig. 112. — [Faivovich, McDiarmid & Myers, 2013](#): 51.

Boana liliae — [Dubois, 2017](#): 28.

Diagnosis: *Myersiohyala liliae* can be diagnosed by the following combination of characters: (1) small snout–vent length in males (SVL 32.5–37.1 mm; females unknown); (2) granular skin on dorsum; (3) presence of an ulnar fold on forearm; (4) single, dark-coloured nuptial pad on Finger II, at the level of the subarticular tubercle between Metacarpal II and Proximal Phalanx; (5) overall coloration green with melanophores homogeneously distributed on the dorsum, ventral surfaces blue, translucent in the central portion of abdomen; in preservative all surfaces become whitish; (6) white parietal peritoneum; (7)

advertisement call composed of a sequence of notes that increase in intensity and rate, as the interval between notes diminishes; (8) dominant frequency of the notes 3.24–3.94 kHz (Kok, 2006).

Characterization: See Kok (2006) for a thorough description of the type series.

Comparison with other species of Myersiohyala: The SVL in males of *M. liliae* (32.5–37.1 mm; Kok, 2006) distinguishes it from all other species of the genus, which are larger (combined SVL of males of *M. aromatica*, *M. chamaeleo*, *M. inparquesi*, *M. loveridgei* and *M. neblinaria* 42–52.3 mm; Rivero, 1961, 1972; Ayarzagüena & Señaris, 1994; Faivovich et al. 2013). The dorsal granular skin distinguishes *M. liliae* from all the other species of *Myersiohyala* (dorsal skin smooth; Rivero, 1961, 1972; Ayarzagüena & Señaris, 1994; Kok, 2006; Faivovich et al., 2013). The presence of an ulnar fold on the forearm distinguishes *M. liliae* from *M. chamaeleo* (ulnar fold absent in this species; Kok, 2006; Faivovich et al., 2013). Whereas *M. liliae* has a single nuptial pad on Finger II (Fig. 6D), *M. aromatica*, *M. chamaeleo* and *M. loveridgei* have two pads (Ayarzagüena & Señaris, 1994; Kok, 2006; Faivovich et al., 2013), and *M. inparquesi* and *M. neblinaria* have a single, larger nuptial pad, which covers dorsally the prepollex and Finger II (Ayarzagüena & Señaris, 1994; Faivovich et al., 2013). The only other species reported to present an overall greenish coloration is *M. chamaeleo*, which also has stellated melanophores (Faivovich et al., 2013). The melanophores in *M. liliae* are less concentrated and more homogeneously distributed than in *M. chamaeleo* (Fig. 6A, B; compare with figs 4 and 7 in Faivovich et al., 2013). The remaining species of *Myersiohyala* have a brownish dorsum that can be marbled with copper (*M. aromatica*), or darker hues (*M. loveridgei*), or have thin black reticulations (*M. inparquesi*), or spots (*M. neblinaria*; Rivero, 1961, 1972; Ayarzagüena & Señaris, 1994; Faivovich et al., 2013). The white peritoneum is shared only with *M. chamaeleo*; *M. neblinaria* has a translucent peritoneum (Faivovich et al., 2013). This character state is unknown in the other species of this genus. Finally, species of *Myersiohyala* with described vocalizations (all but *M. loveridgei*) have calls with a long series of repeated notes; however, the call of *M. liliae* has an increase in both intensity and rate of the call, while *M. aromatica*, *M. chamaeleo*, *M. inparquesi*, and *M. neblinaria* have a call with a constant interval between notes, and constant intensity (Ayarzagüena & Señaris, 1994; Kok, 2006; Faivovich et al., 2013). The dominant frequency of the advertisement call of *M. liliae* (3.24–3.94 kHz; Kok, 2006) is higher than in the other *Myersiohyala* species for which the advertisement call

is known (combined values of dominant frequency of *M. aromatica*, *M. chamaeleo*, *M. inparquesi* and *M. neblinaria* 1.52–2.2 kHz; Ayarzagüena & Señaris, 1994; Faivovich et al., 2013).

Natural history: Specimens of *Myersiohyala liliae* were collected calling from water-filled phytotelms of the bromeliad *Brocchinia micrantha* (Baker, 1879), but in a nearby locality males were heard calling from high elevation in trees close to a field of bromeliads of the same species (Kok, 2006). Whether the species actually reproduces in phytotelms remains unknown. Although the exact place of oviposition is unknown in the other species of *Myersiohyala*, tadpoles of four species have been collected in streams (Ayarzagüena & Señaris, 1994; Faivovich et al., 2013). *Myersiohyala aromatica* and *M. chamaeleo* have been reported to call from bromeliads, close to streams, and *M. neblinaria* at least uses bromeliads for day retreat (Ayarzagüena & Señaris, 1994; Faivovich et al., 2013). Five species of *Myersiohyala* are known to perch on vegetation around streams in the flat-topped tepuis in southern Venezuela, at elevations above 900 m a.s.l. (Rivero, 1961, 1972; Ayarzagüena & Señaris, 1994; Faivovich et al., 2013). *Myersiohyala liliae*, however, is an inhabitant of primary forests at lower elevations (400–550 m a.s.l.; Kok, 2006). There are observations of release of strong odors by *M. aromatica* and *M. inparquesi* (Ayarzagüena & Señaris, 1994); none was noticed by the collectors in *M. liliae* (PJR Kok and BP Noonan, pers. obs.). See Kok (2006) and Kok & Kalamandeen (2008) for more information.

Distribution: This species is known only in western Guyana, where it has been collected in the Potaro-Siparuni District (the Kaieteur Plateau), and in the Cuyuni-Mazaruni District (Imbaimadai). Kok (in Kok & Kalamandeen, 2008) reported hearing calls from this species on the slopes of the Maringma-tepui at the Guyana–Brazil border, and suggested that the species is probably widespread in the Pakaraima (also spelled Pacaraima) Mountains of Guyana.

Remarks: In the original description of *Boana liliae*, Kok (2006) tentatively assigned the new species to *Boana* (as *Hypsiboas*) and to the *B. punctata* group, on the basis of its similarity with *B. cinerascens*, although stressing that there were no putative synapomorphies for this association. Our results, instead, recover a strongly supported sister-taxon relationship with *Myersiohyala chamaeleo*, supporting the association of that species with the genus *Myersiohyala*. This placement, based on our molecular data, actually implies no incongruence with phenotypic evidence.

Kok (2006) characterized *Boana liliae* on the basis of the combination of 22 characters: (1) medium size

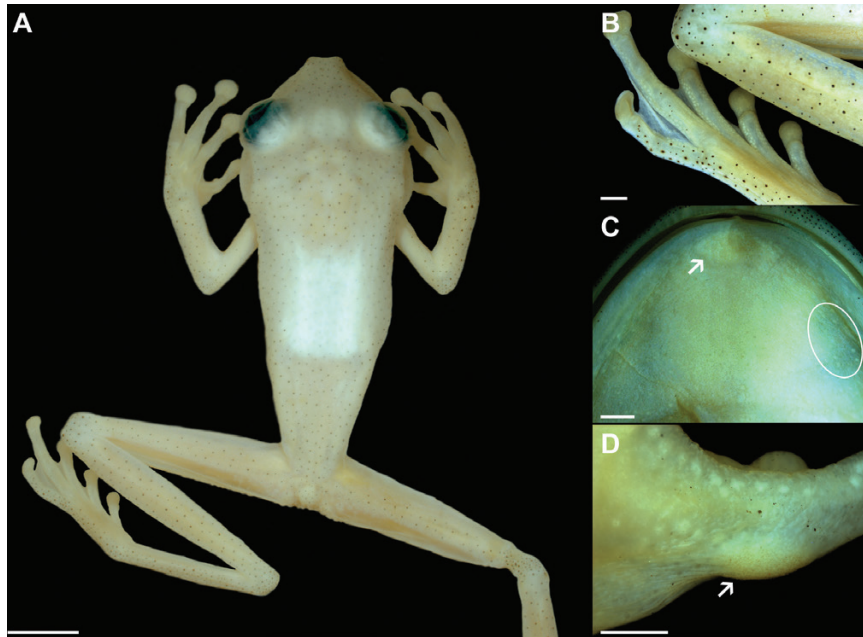


Figure 6. *Myersiohyla liliae*, male paratype (IRSNB 1968). A, dorsal view showing the homogeneous distribution of melanophores. Scale bar 5 mm. B, detail of left shank and feet in dorsal view showing the melanophores on shank and external margin of feet. Note the cumuli of glandular acini (white dots) on toe margins. Scale bar 1 mm. C, ventral view of the mental region. White arrow points the muscle submentalis evident through the skin, white oval surrounds some scattered glandular acini. Scale bar 1 mm. D, dorsal view of left Finger II showing the single nuptial pad (white arrow) at the level of the subarticular tubercle. The dark coloured stratum corneum probably fell due to preservation (see Kok, 2006: fig. 4). Scale bar 0.5 mm.

(SVL 32.5–37.1 mm in adult males; females unknown); (2) skin of dorsum and belly thickly granular; (3) body slender; (4) head slightly wider than long, wider than body; (5) snout truncate in dorsal view and slightly protruding in lateral view, with strongly protuberant nostrils; (6) eyes large, prominent, palpebral membrane lacking reticulations; (7) tympanum large, round, approximately half the horizontal diameter of the eye; (8) supratympanic fold strongly visible, not or feebly obscuring the upper margin of the tympanum; (9) limbs long and slender; (10) axillary membrane absent; (11) subarticular tubercles on fingers single; (12) prepollex enlarged, not modified as a projecting spine; (13) nuptial pads present in males; (14) mental glands in males, small; (15) hands about one-fifth webbed, feet about four-fifths webbed; (16) ulnar fold distinct; (17) inner tarsal fold weak, tarsal tubercles absent; (18) heel tubercles and calcar absent; (19) cloacal sheath absent or very short; (20) in life, dorsal surfaces bright green to bright yellowish green during the day, greenish brown at night, ventral surfaces blue, translucent in the central portion of abdomen, iris silver with black periphery during the day, bronze at night; in preservative all surfaces become whitish; (21) peritoneum white; (22) advertisement call consisting of a long series of loud percussive notes gradually

increasing in speed and loudness (call length about 60 s, up to seven notes per second).

Most of these characters would allow an association with many species in most genera of Cophomantini. A few of these, however, require comment. An enlarged prepollex not modified as a projecting spine [Character (Ch.) 12] is shared with *Nesorohyla*, *Myersiohyla*, *Hyloscirtus* (except *H. condor*, *H. diabolus* Rivera-Correa, García-Burneo & Grant, 2016 and *H. tapichalaca*), *Aplastodiscus* and some species of the *Boana semilineata* group [*B. diabolica* (Fouquet, Martinez, Zeidler, Courtois, Gaucher, Blanc, Lima, Souza, Rodrigues & Kok, 2016), *B. geographica* (Spix, 1824), *B. hutchinsi* (Pyburn & Hall, 1984) and *B. semilineata* (Spix, 1824); Faivovich *et al.*, 2006; Fouquet *et al.*, 2016]. Nuptial pads (Ch.13) occur in *Myersiohyla*, *Nesorohyla*, one species of *Aplastodiscus*, some species of *Bokermannohyla*, *Hyloscirtus* and the *Boana semilineata* species group (Lutz, 1950; Ayarzagüena & Señaris, 1994; Faivovich *et al.*, 2006, 2013; Leite, Pezzuti & Drummond, 2011; Coloma *et al.*, 2012; Rivera-Correa & Faivovich, 2013). The presence of a mental gland in males (Ch. 14) is shared with several species of *Boana*, and also many species of *Aplastodiscus*, *Bokermannohyla*, *Hyloscirtus* and *Myersiohyla* (see Brunetti *et al.*, 2014). The re-examination of the type

series of *M. liliae* shows the occurrence of several glandular acini in the mental region (Fig. 6C; IRSNB 1968). Cumuli of glandular acini were also found in other parts of the body, such as the margins of fingers and toes (Fig. 6B; IRSNB 1968): a histological analysis is required to corroborate whether these skin glands are sexually dimorphic. White peritonea (Ch. 21), the presence of iridophores on parietal or visceral peritonea, are reported in *Aplastodiscus*, some species of *Boana* of the *B. benitezi*, *B. faber*, *B. pellucens*, *B. pulchella* and *B. punctata* groups, the *Hyloscirtus bogotensis* group and *Myersiophyla chamaeleo* (Duellman, 1971; Lutz, 1973; Hoogmoed, 1979; Ruiz-Carranza & Lynch, 1991; Garcia, 2003; Faivovich *et al.*, 2005, 2006, 2013; Berneck *et al.*, 2016). The advertisement call composed of a long series of notes (Ch. 22), as discussed above, is shared with all species of *Myersiophyla* for which calls have been described. In fact, to our knowledge, in *Boana* a similar call structure is present only in *B. faber* (Wied-Neuwied, 1821), *B. pellucens* (Werner, 1901) and *B. boans* (Linnaeus, 1758) (*B. faber*, *B. pellucens*, and *B. semilineata* groups, respectively; Martins & Haddad, 1988; Duellman, 2005).

Our specimens of *M. liliae* are from the Kaieteur Plateau (Potaro-Siparuni District, Guyana), and from Imbaimadai (Cuyuni-Mazaruni District, Guyana). The two localities are 110 km distant from each other, and the specimens have *p*-distances in 16S of 2.2–2.3% (Table 1). This is a considerable molecular intraspecific variation for the ribosomal gene 16S (see Fouquet *et al.*, 2007), and suggests that variation in this species requires an assessment.

DISCUSSION

The two putative autapomorphies of *Nesorohyla kanaima*, black iris in adults and mature oocytes entirely pigmented, without a noticeable external difference between animal and vegetal poles (Duellman & Hoogmoed, 1992; Faivovich *et al.*, 2013), are infrequent in Hylidae. The iris in the other species of *Myersiophyla* has been described as black with metallic copper reticulation in *M. chamaeleo* and *M. neblinaria*, bronze in *M. inparquesi*, silver to bronze in *M. liliae* and grey in *M. loveridgei* (Rivero, 1972; Ayarzagüena & Señaris, 1994; Kok, 2006; Faivovich *et al.*, 2013). It has been described as dark brown in *M. aromatica* (Ayarzagüena & Señaris, 1994), without other details. It would be necessary to find out how well this description actually corresponds with what is described for *N. kanaima*. In the same way, the inclusion of *M. aromatica* in a phylogenetic context will allow testing the polarity of the black iris in adults of *N. kanaima*.

The entirely pigmented ovum is a rare character state. Normally, anuran eggs have an animal pole pigmented with melanin, and an unpigmented vegetal pole; less frequently, an unpigmented animal pole. Entirely pigmented eggs have also been described in *Bufo bufo* (Linnaeus, 1758) and *Leptophryne borbonica* (Tschudi, 1838) (Bufonidae; Boulenger, 1898; Berry, 1972; Iskandar, 1998), *Centrolene geckoideum* Jiménez de la Espada, 1872 (Centrolenidae: Rueda-Almonacid, 1994), *Crossodactylodes itambe* Barata, Santos, Leite & Garcia, 2013 (Leptodactylidae; Santos *et al.*, 2017) and *Odorrana bacboensis* (Bain *et al.*, 2003) (Ranidae; Bain *et al.*, 2003). Whereas both Duellman & Hoogmoed (1992) and Faivovich *et al.* (2013) reported large, mature and entirely pigmented eggs for specimens of *N. kanaima* from Mt. Kanaima and Mt. Roraima, MacCulloch & Lathrop (2005) reported 14 females with black and white oocytes, 1 mm in diameter—a size that may indicate that these oocytes were not yet mature—and four females with tiny, completely white oocytes.

The corroboration that the tadpoles assigned to *Nesorohyla kanaima* by MacCulloch & Lathrop (2005) were properly identified has implications for our understanding of larval evolution in Cophomantini, as they differ from other tadpoles of early diverging lineages of the tribe. These differences include a reduced LTRF (2/4 in *N. kanaima*, versus a minimum of 4/7 but up to 16/21 in known tadpoles of *Myersiophyla*; from 3/4 to 17/19 in *Hyloscirtus*; Sánchez, 2010), short anterior and posterior gaps in the marginal papillae (continuous marginal papillae in known tadpoles of *Myersiophyla* and *Hyloscirtus*; Sánchez, 2010; Coloma *et al.*, 2012; Faivovich *et al.*, 2013) and an oral disc that is apparently not noticeably enlarged, as described in *Myersiophyla* (combined width of oral disc 86–89% of maximum body width; Faivovich *et al.*, 2013) and some species of *Hyloscirtus* (combined width of oral disc 80–90% of maximum body width; La Marca, 1985; Lötters *et al.*, 2005).

Faivovich *et al.* (2005) noted that there was a clear increase in the number of labial tooth rows in the hypothetical ancestor of Cophomantini with respect to the plesiomorphic hylid labial tooth row formula of 2/3, noting at that point a minimum formula of 6/7, but noticing that the minimum values were difficult to establish as the tadpoles of *N. kanaima* (then in *Myersiophyla*) were still unknown. With this information now available, we can infer at least a minimum of four posterior labial tooth rows in the tadpole of the hypothetical ancestor of Cophomantini. Regarding the number of anterior tooth rows, the ancestral state may have been the plesiomorphic two anterior tooth rows, if the number of rows is treated as an additive character state. The inference of the number of posterior

Table 1. Uncorrected *p*-distances between 16S partial sequences of *Myersiophyla liliae*, *M. chamaeleo*, *M. neblinaria* and *Nesorohyla kanaima*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 <i>M. liliae</i>	-															
UTA-A63335																
2 <i>M. liliae</i>	0.00	-														
UTA-A63336																
3 <i>M. liliae</i>	2.16	2.16	-													
IRSNB1968																
4 <i>M. liliae</i>	2.35	2.35	0.54	-												
IRSNB1965																
5 <i>M. chamaeleo</i>	10.70	10.70	10.98	11.19	-											
USNM562057																
6 <i>M. chamaeleo</i>	10.86	10.86	11.14	11.34	0.18	-										
USNM562061																
7 <i>M. chamaeleo</i>	11.01	11.01	11.28	11.49	0.26	0.18	-									
USNM562718																
8 <i>M. chamaeleo</i>	11.01	11.01	11.28	11.48	1.28	1.10	0.91	-								
USNM562056																
9 <i>M. chamaeleo</i>	11.01	11.01	11.28	11.48	1.28	1.10	0.91	0.00	-							
USNM562723																
10 <i>M. neblinaria</i>	13.76	13.76	13.87	14.09	12.79	12.95	13.09	13.64	13.64	-						
USNM562072																
11 <i>M. neblinaria</i>	13.57	13.57	13.69	13.90	12.61	12.76	12.91	13.45	13.45	0.18	-					
USNM562074																
12 <i>M. neblinaria</i>	13.57	13.57	13.69	13.90	12.61	12.76	12.91	13.45	13.45	0.18	0.00	-				
USNM562071																
13 <i>M. neblinaria</i>	13.57	13.57	13.69	13.90	12.61	12.76	12.91	13.45	13.45	0.18	0.00	0.00	-			
USNM562732																
14 <i>N. kanaima</i>	18.84	18.84	18.06	17.53	15.70	16.01	16.15	16.51	16.51	16.80	16.61	16.61	16.61	-		
ROM39582																
15 <i>N. kanaima</i>	20.18	20.18	19.77	19.24	16.68	16.99	17.13	17.49	17.49	18.35	18.17	18.17	18.17	1.80	-	
IRSNB16753																
16 <i>N. kanaima</i>	20.36	20.36	19.95	19.43	16.87	17.18	17.32	17.69	17.68	18.35	18.17	18.17	18.17	1.98	0.18	-
IRSNB16751																

tooth rows is dependent on the phylogenetic position of Cophomantini. So far, most studies (Faivovich *et al.*, 2005; Wiens *et al.*, 2010; Duellman *et al.*, 2016) have recovered Cophomantini as the sister taxon of the other hyline tribes, at least three nodes distant from Lophohylini (a tribe whose phenotypic synapomorphy is a minimum of four posterior labial tooth rows; Faivovich *et al.*, 2005), thus supporting the independent evolution of four posterior labial tooth rows from a plesiomorphic state of three posterior tooth rows.

The phylogenetic position of *Nesorohyla kanaima* introduces an ambiguity in the ancestral character state reconstruction of the extent of occurrence of marginal papillae in the oral disc of the common ancestor of Cophomantini, as a gap could be absent (as in *Hyloscirtus* and *Myersiohyla*; Sánchez, 2010; Faivovich *et al.*, 2013), or present and short (as in *N. kanaima*). Recognizing that the delimitation among states describing the width of the gap may be difficult, the plesiomorphic character state for Hyliinae is the occurrence of a wide anterior gap in the marginal papillae, as in the context of the most recent and complete hypotheses of hylid relationships (Pyron, 2014; Duellman *et al.*, 2016) it optimizes in the common ancestor of Hyliidae (for taxonomic distribution of this character state in Pelodryadinae, Phyllomedusinae and in basal genera of the Hyliinae tribes, see: Kenny, 1969; Duellman, 1970; Tyler & Davies, 1978, 1979; Duellman & de Sá, 1988; Izecksohn, 1996; Faivovich *et al.*, 2010; Sánchez, 2010; Anstis, 2013; Altig & McDiarmid, 2015; Araujo-Vieira *et al.*, 2015; Magalhães, Juncá & Garda, 2015; Pezzuti *et al.*, 2016).

Since the definition of the *Boana punctata* group by Faivovich *et al.* (2005), and the subsequent inclusion of *B. liliae* by Kok (2006), this is the second time that the group has required changes of its species' composition to preserve its monophyly. The first was the transference of *B. ornatissima* to the *B. benitezi* group (Faivovich *et al.*, 2013), and now the transference of *B. liliae* to *Myersiohyla*. Faivovich *et al.* (2013) also called attention to the relationships of *Boana sibleszi*, which was recovered out of the *B. punctata* group, as the poorly supported (62% jackknife) sister taxon of the *B. semilineata* group, as corroborated by our results. At the same time, the monophyly of *B. picturata*, *B. cinerascens* and *B. punctata* remains poorly supported in our analyses, and four species of the group have yet to be included in phylogenetic analyses: *B. alemani*, *B. atlantica*, *B. hobbsi* and *B. jimenezi*. The inclusion of those taxa is necessary for a more stringent test of the monophyly and relationships of the group, and the relationships among species groups of *Boana*.

The phylogenetic relationships of *Myersiohyla liliae* and *Nesorohyla kanaima* have implications for the biogeography of the earlier diverging lineages

of Cophomantini. Most species of *Myersiohyla* and *N. kanaima* are Guiana Shield highland inhabitants (Kok, 2006; Faivovich *et al.*, 2013), the only exception being *M. liliae*. Bearing in mind that the three species of *Myersiohyla* still missing from our phylogenetic analyses (*M. aromatica*, *M. inparquesi*, and *M. loveridgei*), are also Guiana Shield highland inhabitants, the current hypothesis supports the idea that *M. liliae* represents a colonization event of lowland forest by *Myersiohyla*. The poorly supported position of *N. kanaima*, recovered alternatively as the sister taxon of *Myersiohyla* or as the sister taxon of the common ancestor of *Aplastodiscus*, *Boana*, *Bokermannohyla* and *Hyloscirtus*, implies different scenarios. The first topology implies a single Guiana Shield highland origin of the earlier diverging clade of Cophomantini. In the second topology, it is equally parsimonious a highland origin of the common ancestor of Cophomantini, with subsequent diversification in other regions in the sister taxon of *Nesorohyla*, or two independent origins in the Guiana Shield highlands by *Myersiohyla* and *Nesorohyla*.

A pattern of Guiana Shield lowland species that originate from highland clades, as it occurs in *Myersiohyla*, has been noticed in the hemiphraetid *Stefania* (Kok *et al.*, 2012, 2017), the hylid *Tepuihyla* (Kok *et al.*, 2012; Jungfer *et al.*, 2013) and the aromobatid *Anomaloglossus* (Vacher *et al.* 2017). This is not a general pattern in Guiana Shield highland anurans, and Kok (2013) and Kok *et al.* (2012, 2018) presented examples of other scenarios, suggesting an intricate pattern of multiple non-exclusive processes to explain diversification in the region.

ACKNOWLEDGMENTS

We thank R.D. MacCulloch and R. Murphy (Royal Ontario Museum), and R.W. McDiarmid and W. Ron Heyer (United States National Museum) for the loan of specimens. Tiago L. Pezzuti kindly read the ms and provided useful comments. PDPP thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the fellowship at Programa de Pós-Graduação em Ciências Biológicas (Zoologia) at Universidade Estadual Paulista #158681/2013-4; DBM thanks Guyana Environmental Protection Agency for the Permits to Conduct Biodiversity Research (#100703BR009; #011206BR065; #120707BR075; #061212BR020) and for the Permits to Export Biological Specimens for Taxonomic Analysis (#030310SP001; #151206SP030; #1230707SP008; #072412SP054). CFBH thanks CNPq for a research fellowship. JF thanks Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) PICT 2013-404, 2015-820, and grants #2012/10000-5

and #2013/50741-7, Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). PJK's work is supported by postdoctoral fellowships from the Fonds voor Wetenschappelijk Onderzoek Vlaanderen (FWO12A7614N/FWO12A7617N). PJK's fieldwork was supported by the Belgian Directorate-General for Development Cooperation and the King Léopold III Fund for Nature Exploration and Conservation. BPN's fieldwork was supported by the National Science Foundation (DEB-0206562) and National Geographic (7509-03).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

S1. GenBank accession numbers for sequences employed in this study.

S2. Results of the Bayesian analysis. Values at nodes are Posterior Probabilities.