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ORIGINAL ARTICLE

A new terraranan genus from the Brazilian Atlantic Forest with comments on the systematics of Brachycephaloidea (Amphibia: Anura)

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Abstract

"*Eleutherodactylus*" *bilineatus* has long been an enigma. Recent phylogenetic analyses have recovered this species as part of a clade including *Barycholos* and *Noblella*, but the relationship among these groups still remains contentious. In this study, we test the phylogenetic position and reassess the taxonomic status of this long-term *incertae sedis* species. We use phylogenetic analyses of nuclear and mitochondrial gene sequences and data for external morphology and osteology of "*E.*" *bilineatus* and its related genera. We recover the species as an independent lineage forming a fully supported clade with *Barycholos* and *Noblella*. The combination of inferred relationships and morphological traits supports the erection of a new genus that we name and diagnose. Our analyses also recover a non-monophyletic *Noblella* and the species of the genus, although morphologically similar, are part of different clades: one including species from central Andes and the other one including species from northern Andes. Moreover, distribution patterns point out connections among distant biogeographical areas of South America and a widespread distribution of an ancestor for the clade including *Barycholos*, "*E.*" *bilineatus*, and *Noblella*. We also compare the relationships among clades of Brachycephaloidea and, hence, the family and subfamily classifications in different studies. We show that the family classification is probably far from becoming stable, mostly due to arbitrary selections of hierarchy of the clades. However, we show that by assigning a family to each of the highly supported and frequently recovered clades would render a more stable taxonomy of Brachycephaloidea.

KEYWORDS
Barycholos, *Eleutherodactylus bilineatus*, *Noblella*, phylogeny, taxonomy

1 | INTRODUCTION

The species *Eleutherodactylus bilineatus* Bokermann, 1975 has long been an enigma. In its description, Bokermann (1975) clearly stated that the new species was not related to any other known species from eastern Brazil and its relationship to other *Eleutherodactylus* Duméril & Bibron, 1841 was not clear. Lynch (1976) considered that the species could actually be a member of a genus other than *Eleutherodactylus*, since it resembled species of the genus *Adelophryne* Hoogmoed & Lescure, 1984 that were being described by Hoogmoed at that

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time. Nonetheless, after comparing the description of *E. bilineatus* to the material they were studying, Hoogmoed et al. (1994) refused Lynch's (1976) hypothesis and decided to keep Bokermann's generic allocation. *Eleutherodactylus bilineatus* has also been tentatively included in the *Eleutherodactylus fitzingeri* group (Lynch, 1976), but was later considered of unknown affinity and removed from that species group and not assigned to any other species group of the genus (Lynch & Duellman, 1997; Lynch & Myers, 1983).

The evolutionary history of *Eleutherodactylus* remained unknown for a long time. Molecular phylogenetic analyses for a large proportion of the group by Heinicke et al. (2007) found three major clades geographically defined (Caribbean Clade, Middle American Clade, and South American Clade), and a small clade from southeast Brazil represented by two species in the phylogeny— *Eleutherodactylus guentheri* (Steindachner, 1864) and *Eleutherodactylus parvus* (Girard, 1853). Due to its geographic isolation from other groups of *Eleutherodactylus*, 29 species from southeast Brazil, including *E. bilineatus*, were removed from the genus and transferred to the resurrected genus *Ischnocnema* Reinhardt & Lütken, 1862 (Heinicke et al., 2007). Species of *Ischnocnema* were later grouped into five series (*Ischnocnema guentheri*, *Ischnocnema lactea*, *Ischnocnema parva*, *Ischnocnema ramagii*, and *Ischnocnema verrucosa* species series), and *Ischnocnema bilineata* was tentatively added to the *I. lactea* series based on its overall external morphology, since it had not yet been included in phylogenetic analyses (Hedges et al., 2008).

The proposed composition of *Ischnocnema* was long based on poorly sampled phylogenies, as previous studies had included only up to five species out of the more than 30 known species of *Ischnocnema*, and geographic distribution was the main criteria to assign species to the genus (Hedges et al., 2008; Heinicke et al., 2007). Canedo and Haddad (2012) were the first to test the phylogenetic position of *E. bilineatus*. They used a molecular phylogenetic framework composed of 80% of the described species of *Ischnocnema* at the time, including representatives of all species series. Surprisingly, instead of being related to other species from the Atlantic forest of southeast Brazil, *E. bilineatus* was found to be the sister species of a clade including the Andean genus *Noblella* Barbour, 1930 and *Barycholos* Heyer, 1969, a genus that comprises a species from Cerrado and another species from Ecuatorian Chocó (Canedo & Haddad, 2012). However, although *E. bilineatus*, *Barycholos*, and *Noblella* formed a well-supported clade, the relationship of *E. bilineatus* with the other two genera showed low support and the species was left as *incertae sedis* within the subfamily Holoadeninae (Canedo & Haddad, 2012).

Many other phylogenetic analyses further recovered the clade including *Barycholos*, *Noblella*, and "*E.*" *bilineatus* (De la Riva et al., 2017; Guayasamin et al., 2017; Reyes-Puig et al., 2019; Santa-Cruz et al., 2019; Venegas et al., 2018). Nevertheless, the relationship between "*E.*" *bilineatus* and those two genera remains contentious. The species has been placed as either the sister of *Noblella* (De la Riva et al., 2017; Guayasamin et al., 2017; Reyes-Puig et al., 2019; Venegas et al., 2018) or the sister of *Barycholos* and *Noblella* (De la Riva et al., 2017; Santa-Cruz et al., 2019). The low support for the relationship between those two genera and "*E.*" *bilineatus* has prevented taxonomic decisions related to this species. Moreover, in some analyses, the inclusion of "*E.*" *bilineatus* in any of its closest related genera, *Barycholos* or *Noblella*, would render a non-monophyletic grouping. An alternative solution to the inclusion of this species in either *Barycholos* or *Noblella* would be synonymizing *Barycholos* to *Noblella* and include "*E.*" *bilineatus* within *Noblella*. However, this is also not a desirable solution because these two genera are morphologically different, each one having diagnosable morphological characters (Canedo & Haddad, 2012). Guayasamin et al. (2017) found "*E.*" *bilineatus* sister to *Noblella* in both their maximum-likelihood and Bayesian analyses, and besides the low support for this relationship, they placed the species in *Noblella*. Even though it was a new combination, the authors did not discuss or comment on the taxonomic placement of "*E.*" *bilineatus* in *Noblella*.

In this study, we test the phylogenetic position of "*E.*" *bilineatus*, especially regarding its relationship with the Holoadeninae genera *Barycholos* and *Noblella*, and reassess the taxonomic status of this long-term *incertae sedis* species. We use phylogenetic analyses of nuclear and mitochondrial gene sequences and data for external morphology and osteology of "*E.*" *bilineatus* and its related genera to support the recognition of a new genus of Holoadeninae.

2 | MATERIALS AND METHODS

2.1 | Molecular analyses

2.1.1 | Taxon and gene sampling

Morphological examinations were made on 84 specimens; of these, 75 specimens were examined for external morphology and 13 specimens were examined for osteology. Newly produced sequences for 12 specimens and legacy sequences (GenBank) for 143 specimens were included in molecular analysis. We follow the family taxonomy proposed by Padial et al. (2014). According to their classification, "*E.*" *bilineatus*, as well as the genera *Barycholos* and *Noblella*, belong to the subfamily Holoadeninae of the family Craugastoridae. We included all the species belonging to all genera of Holoadeninae that have molecular data available on GenBank, except species of *Qosqophryne* Catenazzi et al., 2020. However, due to the taxonomic instability of families and subfamilies of Brachycephaloidea and the existence of an alternative classification (Heinicke et al., 2018), we included terminals for the other two subfamilies of Craugastoridae. The subfamily Craugastorinae is represented by the genera *Craugastor* Cope, 1862 (*N* = 4), *Haddadus* Hedges et al., 2008 (*N* = 1), and *Strabomantis* Peters, 1863 (*N* = 2), and Ceuthomantinae is represented by *Ceuthomantis* Heinicke et al., 2009 (*N* = 1), *Pristimantis* Jiménez de la Espada, 1870 (*N* = 10), and *Yunganastes* Padial, Castroviejo-Fisher, Köhler, Domic & De la Riva, 2007 (*N* = 2). We also included genera of the family Brachycephalidae, represented by

Brachycephalus Fitzinger, 1826 (*N* = 1) and *Ischnocnema* (*N* = 2), and the family Eleutherodactylidae, represented by *Adelophryne* (*N* = 1), *Diasporus* Hedges et al., 2008 (*N* = 1), and *Eleutherodactylus* (*N* = 1). We rooted all our analyses with *Fritziana fissilis* (Miranda-Ribeiro, 1920) and *Agalychnis callidryas* (Cope, 1862). Our final dataset includes 135 species and 151 terminal taxa.

We chose the mitochondrial 12S rRNA (*12S*), tRNA valine (*tVal*), and partial sequence of 16S rRNA genes (*16S*), as well as nuclear genes recombination-activating gene 1 (*RAG1*) and tyrosinase precursor (*tyr*) to perform our analyses. These gene fragments were available to most of our terminals and have been successfully used in several phylogenetic studies of the Brachycephaloidea (e.g., Canedo & Haddad, 2012; Padial et al., 2014). We produced new sequences for *12S* (*N* = 12), *16S* (*N* = 12), *tVal* (*N* = 12), *RAG1* (*N* = 11), and *tyr* (*N* = 7) genes (Accession Numbers MW201161–MW201176, MW202384–MW202397, MW203017–MW203023) for specimens of *Barycholos ternetzi* (Miranda-Ribeiro, 1937) *(N = 3)*, *Euparkerella cochranae* Izecksohn, 1988 *(N = 2)*, *Euparkerella tridactyla* Izecksohn, 1988 *(N = 2)*, *and "E." bilineatus (N = 5)*. Sequenced specimens of "*E.*" *bilineatus* are from three different localities from the geographical distribution of the species in state of Bahia, Brazil: RPPN Serra Bonita, municipality of Camacan (−15.4413, −39.5189); Fazenda Bonfim, municipality of Uruçuca (−14.6056, −39.3548); and Fazenda Provisão, municipality of Ilheus (−14.6512, −39.2232). Our matrix also includes legacy sequences (GenBank) for *12S* (*N* = 113), *tVal* (*N* = 39), *16S* (*N* = 136), *RAG1* (*N* = 89), and *tyr* (*N* = 94). Specimen voucher numbers and accession numbers for all sequences used in this study are listed in Appendix 1.

2.1.2 | DNA extraction and sequencing

We extracted whole DNA from 99.5% ethanol preserved tissue (muscle or liver) following Lyra et al. (2017) and performed PCR amplifications using Taq DNA Polymerase Master Mix (Ampliqon S/A, Denmark) and Axygen MaxyGene thermocyclers. PCR program was a 3-min initial denaturing step at 95°C, followed by 35–38 (nuclear 42–45) cycles of 20 s at 95°C, 20 s at 50–56°C, and 45 (fragments around 500 bp) or 80 s (fragments around 1,000 bp) at 68°C, followed by a final extension step of 3 min at 68°C. For the *RAG1*, we used a nested-PCR program following Taucce et al. (2018). We built our *12S-tVal-16S* fragment based on three to five fragments of ca. 600 or 1,000 bp each, totaling ca. 2,400 bp. For the nuclear fragments, we targeted 597 bp for *RAG1* and 529–532 bp for *tyr* (primers and respective gene fragments are in Table 1).

2.1.3 | Phylogenetic analyses

We conducted alignment with MAFFT v.730b (Katoh & Standley, 2013) using G-INS-i algorithm for the coding gene fragments (*RAG1* and *tyr*) and the E-INS-i algorithm for the *12S*, *tVal*, and *16S* gene fragments (Alignment S1). We performed an a priori

TABLE 1 Sequence primers used in this study

Primer		Gene	Sequence	Reference
MVZ59	F	*12S*	ATAGCACGTAAAAYGCTDAGATG	Graybeal (1997)
12S L48	F	*12S*	ATGCAAGYMTCMGCRYCCCNGTGA	Walker et al. (2018)
12S F-H	R	*12S*	CTTGGCTCGTAGTTCCCTGGCG	Goebel et al. (1999)
12S A-L	F	*12S*	AAACTGGGATTAGATACCCCACTAT	Goebel et al. (1999)
12S H978	R	*12S*	CTTACCRTGTTACGACTTRCCT	Walker et al. (2018)
12S L13	F	*12S*	TTAGAAGAGGCAAGTCGTAACATGGTA	Feller and Hedges (1998)
16S Titus_1	R	*16S*	GGTGGCTGCTTTTAGGCC	Titus and Larson (1996)
16S L2A	F	*16S*	CCAAACGAGCCTAGTGATAGCTGGTT	Hedges (1994)
16S H10	R	*16S*	TGCTTACGCTACCTTTGCACGGT	Hedges (1994)
16S AR	F	*16S*	CGCCTGTTTATCAAAAACAT	Palumbi et al. (1991)
16S BR	R	*16S*	GACCTGGATTACTCCGGTCTGA	Palumbi et al. (1991)
R182	F	*RAG1*	GCCATAACTGCTGGAGCATYAT	Heinicke et al. (2007)
R270	R	*RAG1*	AGYAGATGTTGCCTGGGTCTTC	Heinicke et al. (2007)
RAG1FF2	F	*RAG1*	ATGCATCRAAAATTCARCAAT	Heinicke et al. (2007)
RAG1FR2	R	*RAG1*	CCYCCTTTRTTGATAKGGWCATA	Heinicke et al. (2007)
Tyr1B	F	*tyr*	AGGTCCTCYTRAGGAAGGAATG	Bossuyt and Milinkovitch (2000)
Tyr1E	R	*tyr*	GAGAAGAAAGAWGCTGGGCTGAG	Bossuyt and Milinkovitch (2000)
Tyr1C	F	*tyr*	GGCAGAGGAWCRTGCCAAGATGT	Bossuyt and Milinkovitch (2000)
Tyr1G	R	*tyr*	TGCTGGGCRTCTCTCCARTCCCA	Bossuyt and Milinkovitch (2000)

Abbreviations: *12S*, 12S rRNA; *16S*, 16S rRNA; *RAG1*, recombination-activating gene 1; *tyr*, tyrosinase precursor.

partition scheme with the three mitochondrial loci and each codon position of the nuclear loci as different partitions, totaling nine partitions. We used PartitionFinder 2.1.1 (Lanfear et al., 2017) to search for the best partition scheme and respective best-fitting nucleotide substitution models under the corrected Akaike information criterion (AICc; Hurvich & Tsai, 1989). PartitionFinder uses a maximum-likelihood software in the analyses, and we chose PhyML 3.0 (Guindon et al., 2010) for this purpose.

We performed tree searches using the Bayesian inference (BI) and maximum likelihood (ML). We computed BI analysis in MrBayes 3.2.6 (Ronquist et al., 2012) using two independent runs of 3.0×10^7 generations, starting with random trees and four Markov chains (one cold), sampled every 3,000 generations. We discarded 25% of generations and trees as burn-in and performed the run with unlinked character state frequencies, substitution rates of the GTR model, gamma shape parameters, and proportion of invariable sites between partitions. We used the standard deviation of split frequencies (<0.01), effective sample size (ESS > 200), and potential scale reduction factor (PSRF; Gelman & Rubin, 1992; should approach 1.0 as runs converge) to assess convergence of the runs. We performed ML analysis in RAxML 8.2.12 (Stamatakis, 2014), searching the most likely tree 100 times, and then, we conducted 1,000 replicates of non-parametric bootstrap to assess support.

2.2 | Morphology

The study of morphology was based on external morphology and osteology. Terminology for morphological characters used in the diagnosis and descriptions follows Duellman and Lehr (2009). Cranial and postcranial osteology follows the terminology in Trueb (1993). We followed Heyer (1975) for the shape of sternum (his character 32), Guayasamin (2004) for the shape of omosternum, and Ponssa (2008) for the shape of extreme of posterolateral process of hyoid (her character 74). The aim of comparisons is to provide evidence of divergence; hence, we restricted comparisons to closely related species, which includes species of *Barycholos*, *Euparkerella* Griffiths, 1959, *Holoaden* Miranda-Ribeiro, 1920, and *Noblella* Northern Clade (sensu Reyes-Puig et al., 2019). Comparisons were based both on descriptions and examination of museum specimens, including types. We analyzed the external morphology of 75 specimens and the osteology of 13 specimens. Specimens examined are listed in Appendix 2. Museum abbreviations are those cited by Frost (2020).

3 | RESULTS

3.1 | Molecular analyses

The final alignment comprises 3,949 bp divided as follows: *12S* (1,118 bp), *tVal* (75 bp), *16S* (1,556 bp), *RAG1* (645 bp), and *tyr* (555 bp). The optimal partition scheme includes eight partitions instead of the nine divided a priori, with the partitions including the second positions of *RAG1* and *tyr* as a single partition. Partitions and respective nucleotide substitution models are in Table 2. In preliminary alignments, the *tVal* fragment of "*E.*" *bilineatus* was only partially homologous to the *tVal* of other brachycephaloids. Because the fragment should be homologous in our whole matrix, we opted to exclude the whole region in "*E.*" *bilineatus* from our analyses.

Our analyses recovered all genera monophyletic, except for *Noblella* and *Psychrophrynella* Hedges et al., 2008, in both ML and BI analyses (Figure 1 and Figure S1). *Noblella madreselva* Catenazzi et al. 2015 is embedded in a clade including *Psychrophrynella chirihampatu* Catenazzi and Ttito, 2016, *Psychrophrynella glauca* Catenazzi and Ttito, 2018, and *Psychrophrynella usurpator* De la Riva et al. 2008, rendering *Psychrophrynella* paraphyletic. The clade including *Noblella losamigos* Santa Cruz et al. 2019, *N. madreselva*, *Noblella pygmaea* Lehr and Catenazzi, 2009, and *Noblella thiuni* Catenazzi and Ttito, 2019 (*Noblella* Southern Clade sensu Reyes-Puig et al., 2019) and *P. chirihampatu*, *P. glauca*, and *P. usurpator* is sister to *Microkayla* De la Riva et al., 2017, while the clade including *Noblella heyeri* (Lynch, 1986), *Noblella lochites* (Lynch, 1976), *Noblella myrmecoides* (Lynch, 1986), *Noblella naturetrekii* Reyes-Puig et al. 2019, and *Noblella personina* Harvey et al. 2013 (*Noblella* Northern Clade sensu Reyes-Puig et al., 2019) is related to *Barycholos* and "*E.*" *bilineatus*.

We recover the clade including *Barycholos*, "*E.*" *bilineatus*, and *Noblella* Northern Clade with ML bootstrap support of 100% (BS = 100) and posterior probability of 100% (PP = 1) in BI. "*E.*" *bilineatus* clearly represents a divergent lineage that is not embedded in *Barycholos* nor in *Noblella* Northern Clade. Moreover, its relationships with *Barycholos* and *Noblella* Northern Clade differ in the ML and BI analyses. In ML, "*E.*" *bilineatus* is sister to *Noblella* Northern Clade with low support (BS = 45) and *Barycholos* is the sister group of this clade. In BI, "*E.*" *bilineatus* is sister to a low-supported clade including *Barycholos* and *Noblella* Northern Clade (PP = 0.82).

TABLE 2 Best partition scheme and respective best-fitting molecular models

Partition	Model
12S	GTR + Γ + I
tVal	GTR + Γ + I
16S	GTR + Γ + I
RAG1 1st position	GTR + Γ + I
RAG1 3rd position	K2P + Γ
tyr 1st position	GTR + Γ + I
tyr 3rd position	SYM + Γ
RAG1 and *tyr* 2nd positions	GTR + Γ + I

Abbreviations: *12S*, 12S rRNA; *16S*, 16S rRNA; GTR, general time-reversible; K2P, Kimura two-parameter; *RAG1*, recombination-activating gene 1; SYM, symmetrical; *tVal*, tRNA valine; *tyr*, tyrosinase precursor.

FIGURE 1 The 50% majority rule consensus tree from Bayesian inference for 135 species and 151 terminal taxa of Brachycephaloidea, based on a dataset of 3,949 aligned bp of fragments of genes 12S rRNA (1,118 bp), tRNA Val (75 bp), 16S rRNA (1,556 bp), recombination-activating gene 1 (645 bp), and tyrosinase precursor (555 bp). Posterior probabilities are indicated at each node (asterisks represent values of 100%). Family and subfamily classifications proposed by Padial et al. (2014) and Heinicke et al. (2018) are presented on the right. We show subfamilies of Craugastoridae sensu Padial et al. (2014) or Strabomantidae sensu Heinicke et al. (2018)

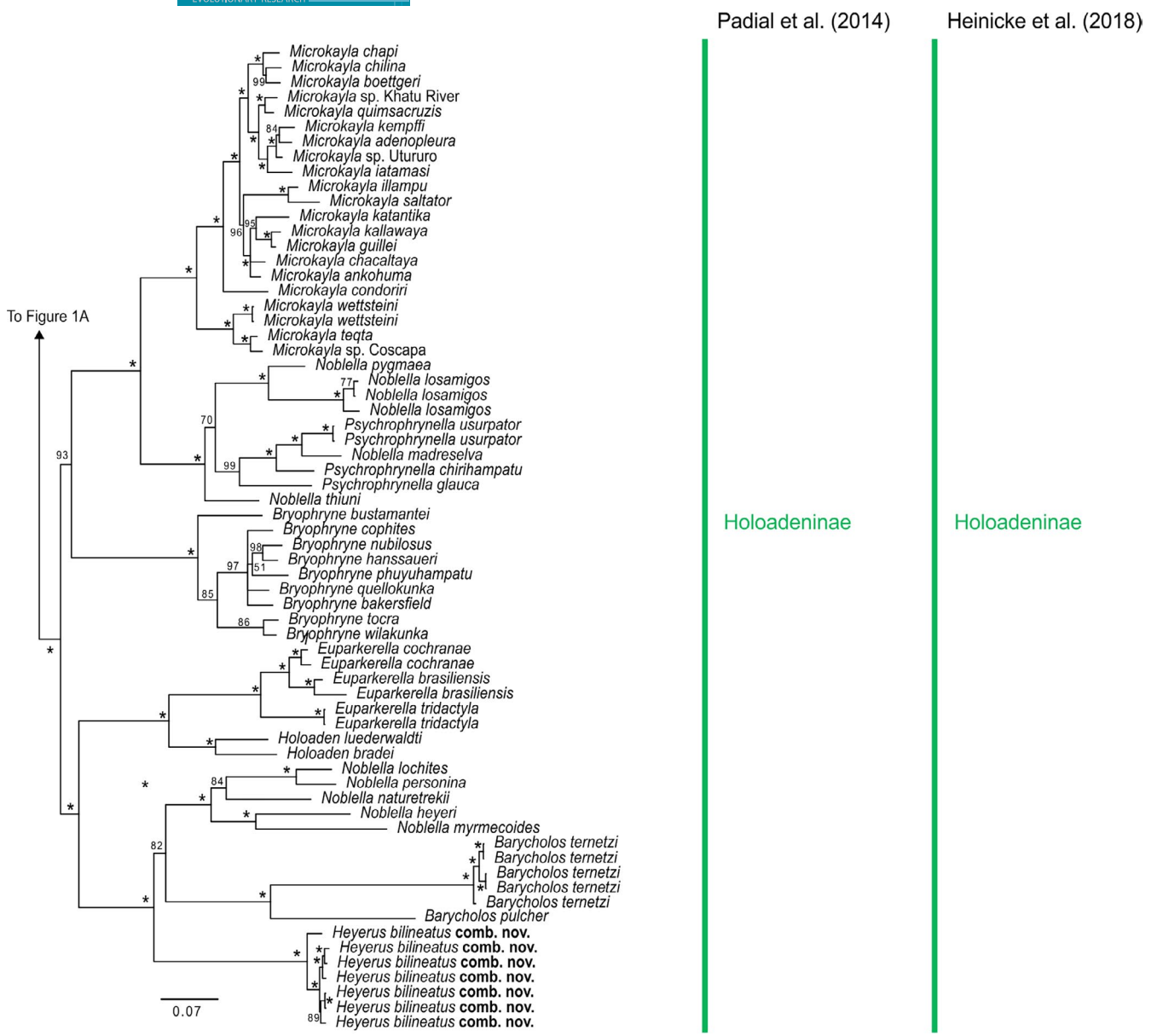

FIGURE 1 (Continued)

3.2 | Systematics

The analysis of external morphology and osteology of "*E.*" *bilineatus* indicates that the species does not belong to either *Barycholos* or *Noblella*. Even though it shares some features that are considered diagnosable for *Barycholos* or *Noblella*, the combination of characters found in "*E.*" *bilineatus* clearly distinguishes it from species of these two genera, as well as from species of the related Atlantic Forest genera *Euparkerella* and *Holoaden*. The phylogenetic position and morphological distinctiveness of "*E.*" *bilineatus* in comparison with its relatives support the placement of this species in a new genus that we name and diagnose.

Order Anura Oppel, 1811

Superfamily Brachycephaloidea Günther, 1858

Family Craugastoridae Hedges et al., 2008

Subfamily Holoadeninae Hedges et al., 2008

Eleutherodactylus bilineatus Bokermann, 1975

Eleutherodactylus (Eleutherodactylus) bilineatus: Lynch & Duellman, 1997

Ischnocnema bilineata: Heinicke et al., 2007

"*Eleutherodactylus*" *bilineatus*: Canedo & Haddad, 2012

Heyerus bilineatus: **New Genus**

Genus *Heyerus* Motta, Taucce, Haddad & Canedo **New Genus**

ZooBank registration: The Life Science Identifier (LSID) for *Heyerus* is urn:lsid:zoobank.org:act:3DC94476-F6CB-4330-BC10-BC0CE8616E29.

Type species: *Heyerus bilineatus* (Bokermann, 1975) by original designation (Figure 2).

Etymology: The specific epithet is used as a noun in the genitive case and honors Dr. W. Ronald Heyer for his invaluable contributions on systematics, evolution, and biogeography of Neotropical amphibians.

FIGURE 2 Adult female holotype (MZUSP 74681) and live specimen of *Heyerus bilineatus*. Photos: MZUSP and Mauro Teixeira Jr

Diagnosis: (a) Skin on dorsum smooth; no dorsolateral folds; (b) tympanic annulus and columella present; tympanic membrane distinct externally; (c) terminal disks on fingers and toes not expanded and not pointed; pads and circumferential grooves present on fingers and toes; terminal phalanges of fingers T-shaped; (d) short, tubercle-like inner tarsal fold present; (e) nasals large, quadrangular, and in contact along their inner border; (f) vomers bearing a broad dentigerous process, separated from each other; vomerine teeth present; anterior alae of vomers broad; (g) alary process of hyoid absent; posterior border of posteromedial process of hyoid expanded and concave (Figure 3); (h) omosternum long and arrow-shaped; sternum bifurcated posteromedially (Figure 4).

Comparisons with other genera: *Heyerus* can be easily distinguished from its closest related genera *Barycholos*, *Euparkerella*, *Holoaden*, and *Noblella* Northern Clade (Table 3). The expanded and concave posterior border of posteromedial process of hyoid (Figure 3) is the only diagnostic character of *Heyerus* that is not shared by any other of those genera. However, that character is not known for any species of *Euparkerella*. The smooth dorsum without folds differentiates *Heyerus* from *Barycholos* (dorsal folds present in *Barycholos*) and *Holoaden* (dorsum heavily glandular in *Holoaden*). The presence of tympanic membrane, tympanic annulus, and columella differentiates *Heyerus* from *Euparkerella* and *Holoaden* (tympanic membrane, tympanic annulus, and columella absent in *Euparkerella* and *Holoaden*). Terminal disks not pointed differ *Heyerus* from *Euparkerella* and *Noblella* Northern Clade whose terminal disks on fingers and toes are pointed. The T-shaped terminal phalanges of fingers differ *Heyerus* from *Euparkerella* (terminal phalanges with hook-like lateral

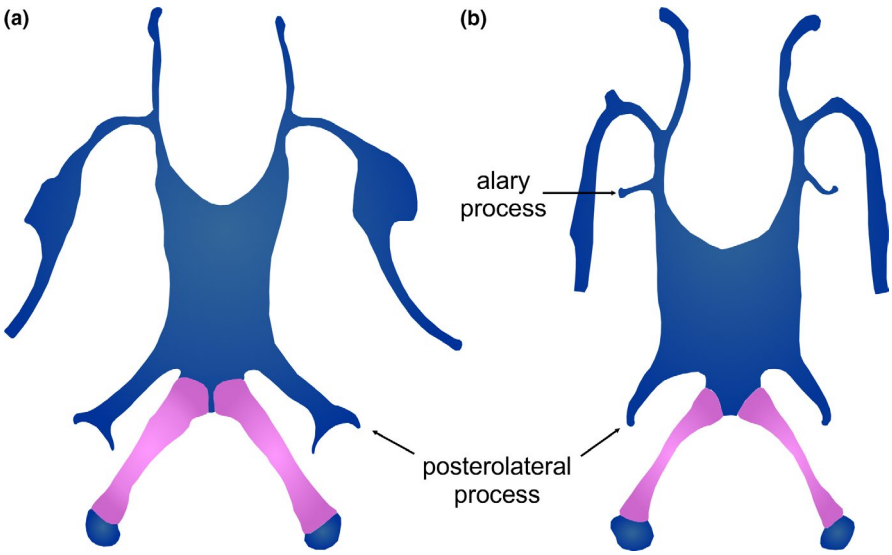

FIGURE 3 Ventral view of hyoid apparatus of (a) *Heyerus bilineatus* (CFBH 35720) and (b) *Barycholos ternetzi* (CFBH 11598). Expanded and concave posterolateral process is represented in (a) and acute posterolateral process is represented in (b). Bones are shown in pink and cartilages are shown in blue

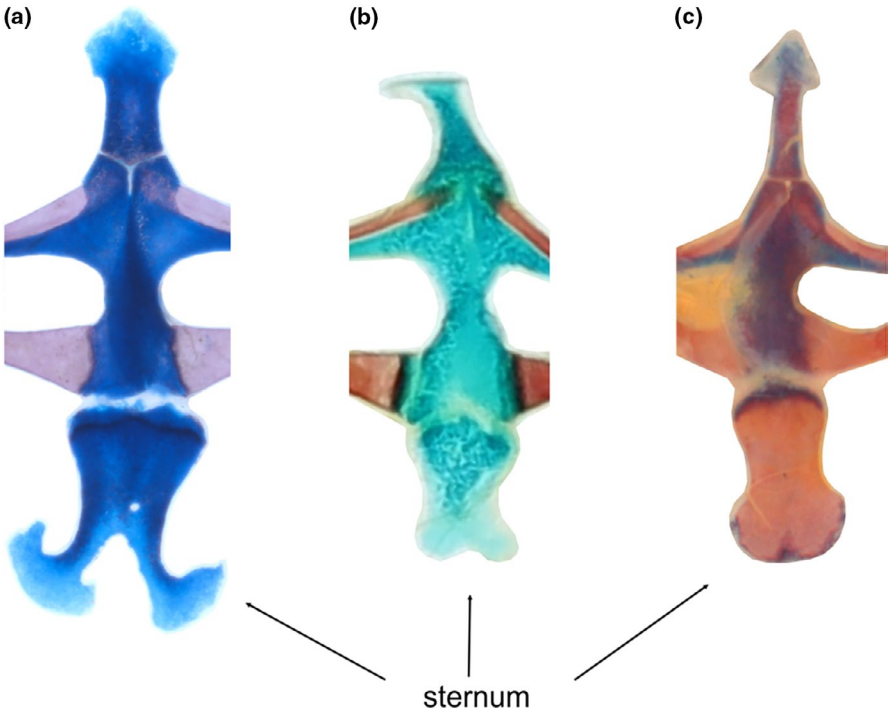

FIGURE 4 Pectoral girdle of (a) *Heyerus bilineatus* (CFBH 35720), (b) *Noblella heyeri* (KU 196531), and (c) *Holoaden luederwaldti* (MNRJ 3903). Sternum bifurcated posteromedially is represented in (a) and (b); sternum not bifurcated is represented in (c). Bones are shown in pink, and cartilages are shown in blue

processes in *Euparkerella*). An inner tarsal fold is present in *Heyerus* and its closest relatives, *Barycholos* and *Noblella* Northern Clade, but it is absent in *Euparkerella* and *Holoaden*. The large quadrangular nasals differentiate *Heyerus* from *Euparkerella*, *Holoaden*, and *Noblella* Northern Clade (nasals are medium-sized in *Euparkerella* and *Holoaden*, and small in *Noblella* Northern Clade). Dentigerous processes and vomerine teeth are absent in *Euparkerella* and *Noblella* Northern Clade and present in *Heyerus*. The absence of alary process of hyoid differs *Heyerus* from *Barycholos* (alary process of hyoid present in *Barycholos*).

Distribution: *Heyerus bilineatus* is known from the Brazilian Atlantic Forest (sea level up to 900 m) from the Paraguaçu River to the Jequitinhonha River in southern and central Bahia, northeastern Brazil (Dias et al., 2017).

4 | DISCUSSION

Molecular and comparative morphological data support the erection of a new monotypic genus of Holoadeninae, Craugastoridae.

TABLE 3 Comparison of characters used to diagnose *Heyerus*

Characters	*Heyerus*	*Barycholos*	*Noblella* Northern clade	*Euparkerella*	*Holoaden*
Skin on dorsum	Smooth	Smooth	Smooth	Smooth	Granular
Dorsolateral folds	Absent	Present	Absent	Absent	Absent
Terminal disk shape	Not pointed	Not pointed	Pointed	Pointed	Not pointed
Terminal phalanges shape	T-shaped	T-shaped	Narrowly T-shaped	Hook-like lateral process	T-shaped
Tarsal fold	Present	Present	Present	Absent	Absent
Nasal size	Large	Large	Small	Medium	Medium
Dentigerous process of vomer	Present	Present	Absent	Absent	Present
Vomerine teeth	Present	Present	Absent	Absent	Present
Alary process of hyoid	Absent	Present	Absent	Absent	Absent
Posterior border of posterolateral process of hyoid	Expanded and Concave	Acute	Acute	-	Acute
Sternum shape	Bifurcated posteromedially	Bifurcated posteromedially	Bifurcated posteromedially	Not bifurcated	Not bifurcated
Tympanic annulus and columella	Present	Present	Present	Absent	Absent

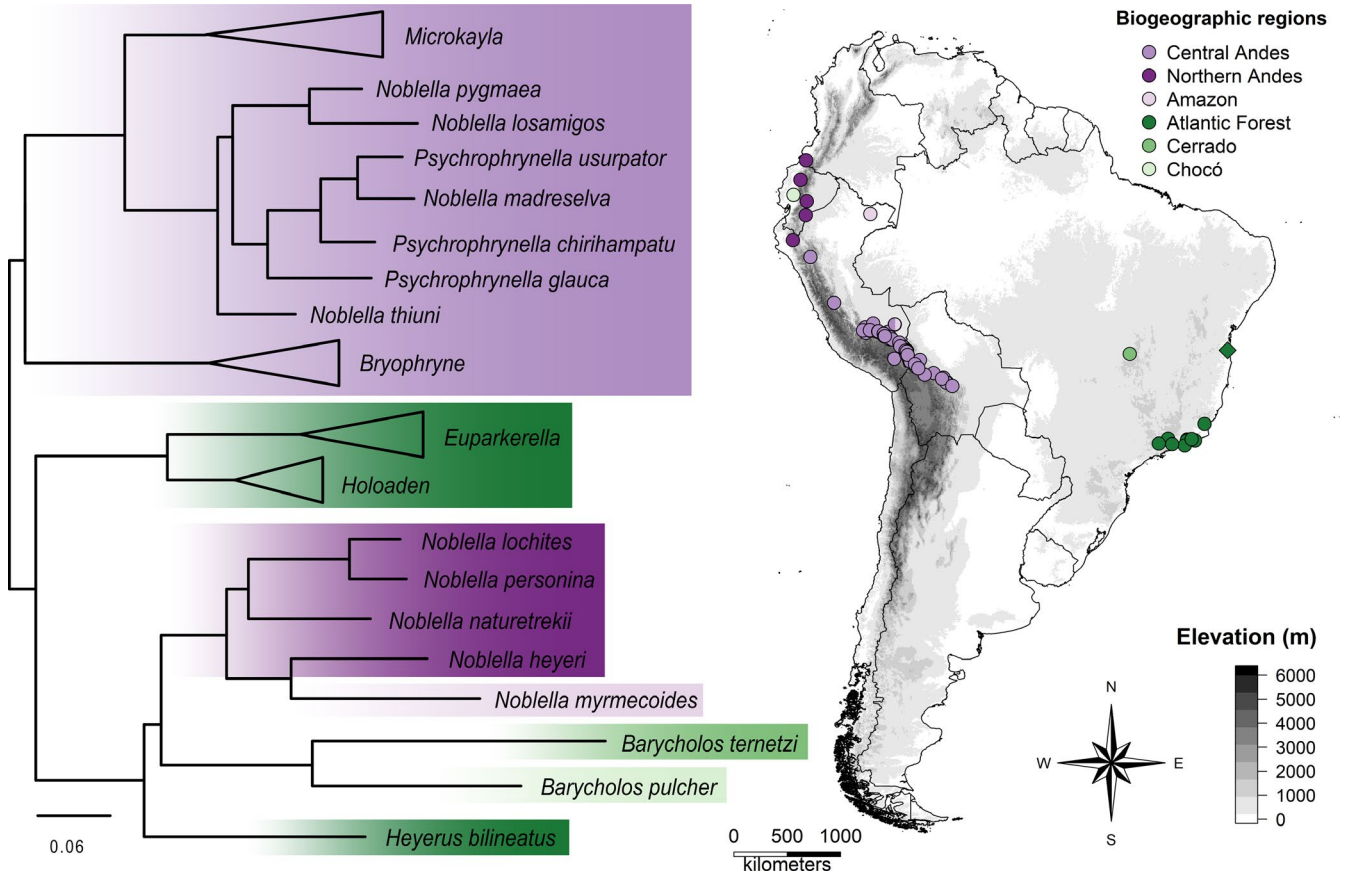

FIGURE 5 The 50% majority rule consensus phylogram and map with type localities of species of the clade including the genera *Barycholos*, *Bryophryne*, *Euparkerella*, *Heyerus*, *Holoaden*, *Microkayla*, *Noblella*, and *Psychrophrynella*. Colors of clades and of points on the map represent habitats where species and clades occur. Diamond shape represents the type locality of *Heyerus bilineatus*

The new genus forms a fully supported clade with *Barycholos* and *Noblella* Northern Clade and shows a combination of morphological traits that distinguishes it from its relatives.

Integrative studies addressing the taxonomy of terraranan frogs have revealed that species believed to form a natural group are actually phylogenetically distant and many new genera of Brachycephaloidea have been recognized and named to accommodate species or a clade of species that represent independent lineages. In their major reorganization of direct-developing frogs, Hedges et al. (2008) recovered and erected many genera to include natural groups of species, and subsequently, four new genera of Brachycephaloidea were described (Catenazzi et al., 2020; De la Riva et al., 2017; Heinicke et al., 2009, 2015). Many of these genera contained less than five species when they were erected or described, and interestingly, subsequent studies revealed a much greater diversity in some of them. For example, *Bryophryne* Hedges et al., 2008 included only two species in its description and is now composed of 11 species (Catenazzi et al., 2020; De la Riva et al., 2017; Hedges et al., 2008) and *Lynchius* was described to accommodate three species and now is composed of seven species (Hedges et al., 2008; Motta et al., 2016; Sánchez-Nivicela et al., 2019). Moreover, Trevisan et al. (2020) found a remarkable hidden diversity in the genus *Pristimantis* from the northern Brazilian Atlantic Forest. Their analyses show that the populations of *Pristimantis* assigned to three nominal species represent nine highly structured lineages that may correspond to undescribed species. In this regard, the diversity of *Heyerus* could be underestimated and future researches might reveal a higher number of species belonging to this genus. On the other hand, terraranan clades show a high heterogeneity in rate of diversification regardless of clades age (González-Voyer et al., 2011) and some genera from the Brazilian Atlantic Forest, such as *Euparkerella*, *Holoaden*, and *Haddadus*, as well as *Barycholos*, represent clades that have low species richness. Therefore, it is possible that *Heyerus* also constitutes a lineage of terraranan frogs with low species diversity, similar to its closely relative genera.

Inferred relationships and distribution patterns point out connections among distant biogeographical areas of South America (e.g., Atlantic Forest and the Andes; Cerrado and Chocó), and a widespread distribution of an ancestor for the clade including *Barycholos*, *Heyerus*, and *Noblella* Northern Clade (Hedges et al., 2008; Figure 5). A Northern Atlantic Forest–Andean connection is also supported by phylogenetic relationships of species in the terraranan genus *Oreobates* Jiménez de la Espada, 1872 (Teixeira Jr. et al., 2012; Vaz-Silva et al., 2018), and *Pristimantis* (Canedo & Haddad, 2012).

Inferred relationships also show that species with similar external morphology from central and northern Andes that have been

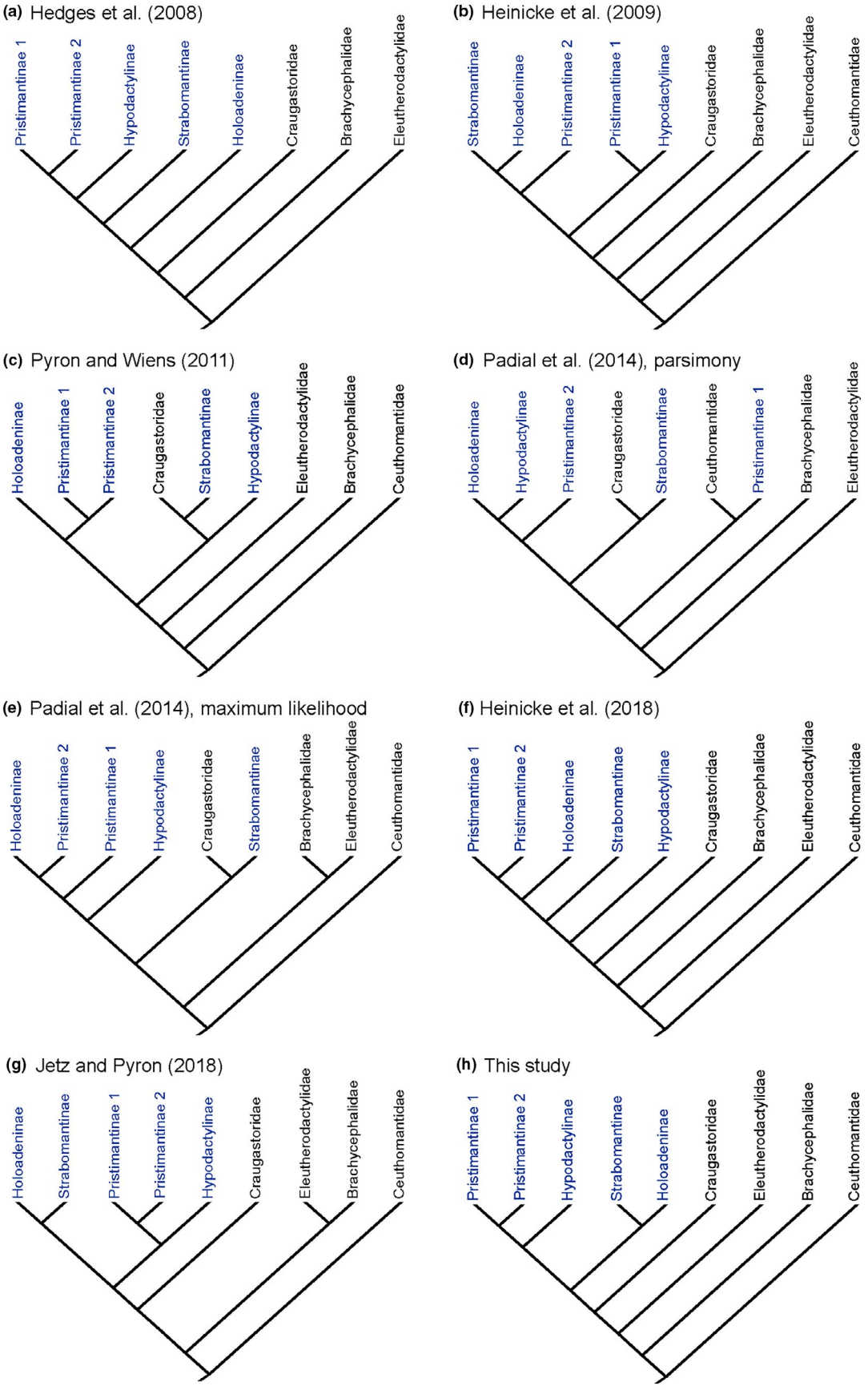

FIGURE 6 Relationships among families and subfamilies of Brachycephaloidea sensu Heinicke et al. (2018). Subfamilies of the family Strabomantidae are highlighted in blue. The clade including *Pristimantis* and *Yunganastes* is represented by Pristimantinae 1, and the clade including *Lynchius*, *Oreobates*, and *Phrynopus* is represented by Pristimantinae 2. Adapted from figure 8 in Padial et al. (2014)

included in the same genus are, in fact, part of different clades. The genus *Lynchius* Hedges et al., 2008, for example, was erected by Hedges et al. (2008) to include three species from northern Andes that were considered part of *Phrynopus* Peters, 1873, an exclusively central Andean genus. Our analyses recover species of *Noblella* in two different clades, one including species from central Andes and the other one including species from northern Andes, that are not sister to each other. Previous studies have found the same relationship (Catenazzi et al., 2020; Catenazzi & Ttito, 2019; De la Riva et al., 2017; Reyes-Puig et al., 2019; Santa-Cruz et al., 2019), but they refrain from proposing a new generic arrangement since the affinities of *Noblella peruviana* (Noble, 1921) and *Psychrophrynella bagrecito* (Lynch 1986), the type species of *Noblella* and *Psychrophrynella*, remain unclear. Morphological evidence indicates that *P. bagrecito*, as well as most species of the genus, share a considerable number of traits with *N. peruviana* (De la Riva et al., 2017). In a scenario where these two species are part of the clade including *Noblella* Southern Clade and *Psychrophrynella* species, *Psychrophrynella* would become a junior synonym of *Noblella*, while *Phyllonastes* Heyer, 1977 would be the name available for the *Noblella* Northern Clade. An alternative scenario would place *N. peruviana* in the *Noblella* Northern Clade. In this case, species of *Noblella* Southern Clade would be transferred to *Psychrophrynella* and the name *Noblella* would apply to the *Noblella* Northern Clade. Therefore, it is crucial to infer the phylogenetic position of the type species *N. peruviana* and *P. bagrecito* before making any taxonomic decision about these genera.

The classification of families and subfamilies of Brachycephaloidea has faced many changes since 2008, when Hedges et al. (2008) rearranged the taxonomy of the group. One reason for this instability is that the relationship among genera varies among the different analyses, and authors have been including in the same family genera from clades that show low support and, therefore, have different relationships in different analyses, rendering proposed families paraphyletic (see Hedges et al., 2008; Heinicke et al., 2018; Padial et al., 2014). Another important reason for the instability is that the decisions have been made based only on molecular data, and synapomorphies for clades representing different hierarchies (family, subfamily, and genus) have not yet been proposed. This makes the selected hierarchy of a clade (i.e., if the clade is considered a family, subfamily or genus) arbitrary and contributes to the taxonomic instability in this group.

However, it is worth noting that, even considering different datasets and optimality criteria, there are clades that show high stability, that is, clades that have been recovered with high support in all available analyses that include representatives of all families of brachycephaloids: *Lynchius*, *Oreobates*, and *Phrynopus*; *Euparkerella* and *Holoaden*; *Pristimantis* and *Yunganastes*; *Barycholos*, *Bryophryne*, *Heyerus*, *Microkayla*, *Noblella*, and *Psychrophrynella*.

Even though most of those stable clades are represented by the subfamilies of Strabomantidae in Heinicke et al. (2018), the family classification is probably far from becoming stable. The instability of the families Craugastoridae and Strabomantidae is mostly due to the position of the genus *Strabomantis*. When *Strabomantis* is recovered as sister group to a clade that comprises *Craugastor*, Strabomantidae is included in the synonym of Craugastoridae (Padial et al., 2014; Pyron & Wiens, 2011). When *Strabomantis* is recovered embedded in the Strabomantidae clade, and the clade comprising *Craugastor* is the sister clade to all other Strabomantidae, the two families are considered valid (Hedges et al., 2008; Heinicke et al., 2018; Jetz & Pyron, 2018) (Figure 6).

A solution to the instability in the family taxonomy of this group would be to split the subfamily Pristimantinae sensu Heinicke et al. (2018) into two families (one including *Pristimantis* and *Yunganastes*, and another including *Lynchius*, *Oreobates*, and *Phrynopus*) and elevate the subfamilies sensu Heinicke et al. (2018) to family rank. That classification would render all the families monophyletic in all available analyses to date that have included representatives of all families of brachycephaloids. Ongoing studies of the anatomy of terraranan frogs (e.g., Taboada et al., 2013) are leading to the discovery of synapomorphies at different levels. In view of the relevance of including morphological datasets for terraranan systematics, we reinforce the need of integration of various data sources for a more accurate classification of this group.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Figure S1. Maximum-likelihood optimal tree of 135 species and 151 terminal taxa of Brachycephaloidea, based on a dataset of 3,949 aligned bp of fragments of genes 12S rRNA (1,118 bp), tRNA Val (75 bp), 16S rRNA (1,556 bp), recombination-activating gene 1 (645 bp), and tyrosinase precursor (555 bp).

Alignment S1. DNA sequences alignment for mitochondrial and nuclear genes used for the phylogenetic analyses.

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APPENDIX 1

GenBank accession numbers of species sampled in this study

Species	Voucher	*12S–tVal–16S*	*RAG1*	*tyr*
Adelophryne gutturosa	ROM 39578	EU186679	EU186751	EU186772
Agaluchnis callidryas	—	DQ283423	EF493362	DQ283018
Barycholos pulcher	KU 217781	EU186727, EU186709	KX208662	EU186765
Barycholos ternetzi	CFBH 19426	JX267466	JX267543	JX267680
Barycholos ternetzi	CFBH 23511	KU495148	KF625108	KF625130
Barycholos ternetzi	CFBH 11596	**MW202396**, **MW202388**	**MW201166**	—
Barycholos ternetzi	CFBH 11597	**MW202397**, **MW202389**	**MW201167**	**MW203017**
Barycholos ternetzi	CFBH 11598	**MW202395**, **MW202390**	**MW201168**	**MW203018**
Brachycephalus ephippium	CFBH 16807	HQ435679, HQ435693	HQ435721	HQ435735
Bryophryne bakersfield	MUBI 6022	MF186284, MF186341	MF186528	—
Bryophryne bustamantei	MHNC 6019	KT276286, KT276293	MF186544	MF186548
Bryophryne cophites	KU 173497	EF493537	EF493423	EF493508
Bryophryne hanssaueri	MUSM 27567	KY652642	KY681084	KY681063
Bryophryne nubilosus	MUSM 27882	KY652643	KY681085	KY681064
Bryophryne phuyuhampatu	CORBIDI 18224	MF419254	—	—
Bryophryne quellokunka	MNCN 43780	MF186309, MF186387	MF186526	—
Bryophryne tocra	MNCN 43786	MF186315, MF186396	MF186541	—
Bryophryne wilakunka	MUBI 5425	MF186291, MF186349	—	—
Ceuthomantis smaragdinus	ROM 40161	EU186677	EU186750	EU186771
Craugastor daryi	UTA-A 57940	EF493531	EF493452	EF493480
Craugastor longirostris	KU 177803	EF493395	EF493454	EF493482
Craugastor pygmaeus	UTA-A 55241	EF493711	EF493451	EF493479
Craugastor spatulatus	AMCC 118375	EU186674	EU186749	EU186770
Diasporus diastema	MVZ 203844	EU186682	EU186752	EU186773
Eleutherodactylus cooki	USNM 326784	EF493539	EF493413	EF493455
Euparkerella brasiliensis	—	JX267390, JX267468	JX267545	JX267682
Euparkerella brasiliensis	—	JX298276, JX298316	JX298185	JX298237
Euparkerella cochranae	MNRJ 56146	**MW202392**, **MW202384**	**MW201175**	KF625114
Euparkerella cochranae	MNRJ 72082	**MW202391**, **MW202385**	**MW201176**	KF625116
Euparkerella tridactyla	JFT 305	**MW202394**, **MW202387**	**MW201173**	KF625109
Euparkerella tridactyla	JFT 346	**MW202393**, **MW202386**	—	KF625110
Fritziana aff. *fissilis*	MNRJ 44622	KR559917, KR270404, KR270421	KR138396	KR270390
Haddadus binotatus	MTR 13438	JX267346	JX267547	JX267684
Heyerus bilineatus	CFBH 23684	**MW201162**	**MW201169**	**MW203019**
Heyerus bilineatus	CFBH 23689	**MW201163**	**MW201170**	**MW203020**
Heyerus bilineatus	CFBH 32419	**MW201164**	**MW201171**	**MW203021**
Heyerus bilineatus	CFBH 34063	**MW201165**	**MW201172**	**MW203022**
Heyerus bilineatus	MNRJ 46476	JX267393, JX267324	JX267556	JX267691
Heyerus bilineatus	MNRJ 52873	JX267323	JX267555	—
Heyerus bilineatus	CFBH 35720	**MW201161**	**MW201174**	**MW203023**
Holoaden bradei	USNM 207945	EF493378, EF493366	EF493449	EU186779
Holoaden luederwaldti	MZUSP 131872	EU186728, EU186710	EU186747	EU186768
Ischnocnema guentheri	CFBH 26993	JX267331, JX267501, JX267502	JX267611	JX267746
Ischnocnema verrucosa	CFBH 23685	JX267457, JX267538	JX267670	JX267810

(Continued)

APPENDIX 1 (Continued)

Species	Voucher	*12S–tVal–16S*	*RAG1*	*tyr*
Lynchius flavomaculatus	KU 218210	EU186667	EU186745	EU186766
Lynchius nebulanastes	KU 181408	EU186704	–	–
Lynchius oblitus	MHNC 8676	KX470778, KX870785	KX470794	KX470801
Lynchius parkeri	KU 181307	EU186705	–	–
Lynchius simmonsi	QZ 41639	JF809940, JF810004	JF809915	JF809894
Lynchius tabaconas	MHCN 8637	KX470773, KX470780	–	KX470796
Microkayla adenopleura	MNCN 44810	MF186283, MF186340	MF186537	MF186565
Microkayla ankohuma	MNK-A 7280	MF186288, MF186346	–	MF186560
Microkayla boettgeri	MUBI 5363	MF186294, MF186353	–	MF186559
Microkayla chacaltaya	MNCN 42052	MF186357	MF186532	–
Microkayla chapi	MNCN 43762	MF186328, MF186417	MF186540	MF186562
Microkayla chilina	MNCN 43772	MF186327, MF186414	MF186539	MF186561
Microkayla condoriri	CBF 5989	MF186300, MF186360	MF186530	MF186550
Microkayla guillei	AMNH-A 165108	AY843720	–	DQ282995
Microkayla iatamasi	MNCN 42054	MF186304, MF186368	MF186536	MF186558
Microkayla illampu	CBF 5998	MF186369	MF186534	MF186549
Microkayla kallawaya	MNCN 42061	MF186376	–	MF186575
Microkayla katantika	CBF 6013	MF186307, MF186381	MF186533	MF186576
Microkayla kempffi	MNCN 43646	MF186308, MF186384	MF186538	MF186566
Microkayla quimsacruzis	MNCN 42063	MF186323, MF186405	–	–
Microkayla saltator	CBF 6033	MF186326, MF186410	–	–
Microkayla sp.	CBF 6564	MF186317, MF186399	–	MF186556
Microkayla sp.	MNCN 46980	MF186332, MF186426	–	MF186568
Microkayla sp.	MNCN 42034	MF186325, MF186409	MF186535	MF186563
Microkayla teqta	MNCN 45702	MF186318, MF186400	–	MF186552
Microkayla wettsteni	–	AM039711, AM039643	–	–
Microkayla wettsteni	CBF 6241	MF186338, MF186434	MF186531	MF186551
Niceforonia brunnea	KU178258	EF493357	GQ345282	EF493484
Niceforonia dolops	–	EF493394	EF493414	EF493483
Niceforonia elassodiscus	KU 178282	EF493358	–	–
Niceforonia nana	IAvHAm 13054	MH532902, MH536808	–	MH542228
Niceforonia nigrovittata	CORBIDI 9547	MH538300	–	–
Niceforonia peraccai	KU 178266	EF493710	EF493420	EF493485
Noblella heyeri	QCAZ 31471	JX267463, JX267541	–	–
Noblella lochites	KU 177356	EU186699	EU186756	EU186777
Noblella losamigos	AC94_09	MN336183	–	–
Noblella losamigos	MUSA 6302	MN100040	–	–
Noblella losamigos	MVZ 292687	MN366392	–	–
Noblella madreselva	CORBIDI 15770	MN056356	–	–
Noblella myrmecoides	QCAZ 40180	JX267464, JX267542	–	–
Noblella naturetrekii	QCAZ 71337	MK838467, MK838462	–	–
Noblella personina	QCAZ 58818	MK838468, MK838465	–	–
Noblella pygmaea	MUSM 24536	KY652645	KY681086	KY681066
Noblella thiuni	CORBIDI 18723	MK072732	–	–
Oreobates amarakaeri	MHNC 6975	JF809934, JF809996	JF809913	JF809891
Oreobates antrum	ZUFG 5888	MH025427, MH025451	MH025436	MH025445

(Continued)

APPENDIX 1 (Continued)

Species	Voucher	*12S–tVal–16S*	*RAG1*	*tyr*
Oreobates ayacucho	MNCN(IDIR5024)	JF809933, JF809970	JF809912	JF809890
Oreobates barituensis	MCN 1359	JF809935, JF809999	JF809914	JF809892
Oreobates berdemenos	FML 24623	KJ125509	—	––
Oreobates choristolemma	CBG 765	JF809921, FJ539072, FJ539067	JF809900	JF809881
Oreobates crepitans	ZUEC 14119	KJ125510	—	—
Oreobates cruralis	KU 215462	EU186666	EU186743	EU186764
Oreobates discoidalis	MNCN 43133	JF809925, FJ539073, FJ539068	—	JF809884
Oreobates gemcare	MHNC 6687	JF809930, JF809960	JF809909	—
Oreobates granulosus	MHNC 3396	JF809929, FJ539074, EU368897	JF809908	JF809887
Oreobates heterodactylus	MNK-A 7175	JF809923, FJ438816, EU192296, FJ438805	JF809902	JF809882
Oreobates ibischi	MNK-A 6612	FJ438817, FJ438806	—	—
Oreobates lehri	MUSM 27616	JF809927, JF809957	JF809906	—
Oreobates lundbergi	MTD 45902	JF809928, JF809958	JF809907	JF809886
Oreobates machiguenga	MHNC 6809	JF809932, JF809969	JF809911	JF809889
Oreobates madidi	MNK-A 7856	JF809922, FJ539075, FJ539070	JF809901	—
Oreobates pereger	MTD 46808	JF809926, JF809955	JF809905	JF809885
Oreobates quixensis	KU 178249	EF493828, EF493662	—	—
Oreobates remotus	MZUSP 141708	JN688273	—	—
Oreobates sanctaecrucis	MNK-A 5507	JF809924, JF809951	JF809903	JF809883
Oreobates sanderi	MNCN 42017	EU368904	—	—
Oreobates saxatilis	KU 212327	EU186726, EU186708	EU186742	EU186763
Oreobates yanucu	ZFMK 72569	KY111322	—	—
Phrynopus auriculatus	MUBI 6471	MF186290, MF186348	—	MF186582
Phrynopus badius	FMNH 282818	MG896594, MG896571	MG896618	—
Phrynopus barthlenae	MHNSM 20609	MF186292, MF186350	—	—
Phrynopus bracki	USNM 286919	EF493709	EF493421	EF493507
Phrynopus bufoides	—	AM039713, AM039645	—	—
Phrynopus daemon	MUSM 32747	MG896597, MG896574	—	—
Phrynopus heimorum	MTD 45621	MF186302, MF186363	MF186545	MF186580
Phrynopus horstpauli	MTD 44335	MF186303, MF186364	—	MF186584
Phrynopus interstinctus	MUSM 29543	MG896598, MG896575	MG896621	—
Phrynopus inti	UMMZ 245218	MF651913, MF651906	MF651918	MF651921
Phrynopus juninensis	MUSM 38324	MG896600, MG896577	MG896623	—
Phrynopus kauneorum	—	AM039718, AM039650	—	—
Phrynopus mariellaleo	CORBIDI 11658	MH538299	—	MH538306
Phrynopus miroslawae	MUBI 6469	MF186312, MF186393	MF186542	MF186585
Phrynopus montium	MUSM 33259	MG896601, MG896578	MG896624	—
Phrynopus peruanus	MUSM 38316	MG896605, MG896582	MG896626	MG896631
Phrynopus pesantesi	—	AM039724, AM039656	—	—
Phrynopus tautzorum	—	AM039720, AM039652	—	—
Phrynopus tribulosus	MUBI 7166	MF186330, MF186424	MF186547	MF186579
Phrynopus unchog	MUSM 32748	MG896608, MG896591	—	—
Phrynopus vestigiatus	MUSM 29542	MG896610, MG896593	—	—
Pristimantis actites	KU 217830	EF493696	EF493432	EF493494
Pristimantis altamazonicus	KU 215460	EF493670	EF493441	—

(Continued)

APPENDIX 1 (Continued)

Species	Voucher	*12S–tVal–16S*	*RAG1*	*tyr*
Pristimantis duellmani	WED 53050/KU 217998	AY326003	EF493438	EF493500
Pristimantis euphronides	BWMC 6918	EF493527	EF493427	EF493489
Pristimantis leoni	KU 218227	EF493684	EF493443	EF493495
Pristimantis nyctophylax	KU 177812	EF493526	EF493425	EF493487
Pristimantis ramagii	MNRJ 36751	JX267318	JX267658	JX267797
Pristimantis rozei	—	EF493691	EF493429	EF493491
Pristimantis stictogaster	KU 291659	EF493704	EF493445	EF493506
Pristimantis versicolor	KU 218096	EF493389	EF493431	EF493493
Psychophrynella usurpator	AC186_09	KY652662	KY672975	KY681083
Psycophrynella chirihampatu	MHNC 14664	KU884560	—	—
Psycophrynella glauca	CORBIDI 18729	MG837565	—	—
Psycophrynella usurpator	KU 173495	EF493714	—	—
Strabomantis biporcatus	CVULA 7073	EU186691	EU186754	EU186775
Strabomantis bufoniformis	SIUC 7062	DQ283165	—	DQ282942
Yunganastes fraudator	MNCN 43107	JF809938	JF809916	JF809895
Yunganastes mercedesae	ZFMK 72571	JF809939	JF809920	JF809899

Sequences produced in this study are highlighted in bold font.

Abbreviations: *12S*, 12S rRNA; *16S*, 16S rRNA; *RAG1*, recombination-activating gene 1; *tVal*, tRNA valine; *tyr*, tyrosinase precursor.

APPENDIX 2

SPECIMENS EXAMINED IN THIS STUDY, INCLUDING CLEARED AND STAINED SPECIMENS (CS)

Specimens in bold were examined for external morphology and osteology. Asterisks indicate specimens we sequenced in this study.

Barycholos pulcher: AMNH 89707, AMNH 89714, AMNH 104345, KU 218156 (CS), KU 218158 (CS); *Barycholos ternetzi*: CFBH 26150, CFBH 23596, CFBH11599, CFBH 26105, CFBH 26108, CFBH 10260, CFBH 11595, CFBH 11600, CFBH 23818, CFBH 11601, CFBH 26038, CFBH 11594, CFBH 11596*, **CFBH 11597*** (CS), **CFBH 11598*** (CS); *Euparkerella brasiliensis*: KU 93192 (CS), CFBH 39336, CFBH 26983, CFBH 253, CFBH 39340, CFBH 31334, CFBH 40840, CFBH 39335, CFBH 25984, CFBH 39333, CFBH 39338, CFBH 39337; *Euparkerella cochranae*: CFBH 272, CFBH 270, CFBH 273, CFBH 271, CFBH 35224, CFBH 274; *Euparkerella tridactyla*: CFBH 948, CFBH 1360, CFBH 950, CFBH 22526*, CFBH 35672, CFBH 33134; *Heyerus bilineatus*: CFBH 23689*, CFBH 34088, CFBH 34063*, CFBH 34090, CFBH 32419*, CFBH 37976, **CFBH 23684*** (CS), **CFBH 35720*** (CS); *Holoaden bradei*: AMNH 73548, AMNH 73549, KU 107087 (CS), KU 92868 (CS), KU 107088 (CS), MNRJ 22501 (CS), CFBH 36357, CFBH 252; *Holoaden luederwaldti*: AMNH 72334, MNRJ 3903 (CS), CFBH 19552, CFBH 19553, CFBH 19554, CFBH 19555, CFBH 19550, CFBH 19551, CFBH 19556, CFBH 29298, CFBH 9900, CFBH 9932, CFBH 9899, CFBH 9914; *Noblella heyeri*: KU 196529, KU 196530, KU 196531 (CS); *Noblella lochites*: KU 147070 and data from Heyer (1977); *Noblella myrmecoides*: AMNH 102997, AMNH 97051, AMNH 153037, and data from Heyer (1977); *Noblella pygmaea*: MUSM 26320, MUSM 24535, MUSM 24536.