Phylogenomic Analyses Reveal Non-Monophyly Of The Antbird Genera Herpsilochmus And Sakesphorus (Thamnophilidae), With Description Of A New Genus For Herpsilochmus Sellowi

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Phylogenomic analyses reveal non-monophyly of the antbird genera *Herpsilochmus* and *Sakesphorus* (Thamnophilidae), with description of a new genus for *Herpsilochmus sellowi*

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**ABSTRACT**

The family Thamnophilidae is a species-rich Neotropical radiation of passerine birds. Current classification of its 235 species is mostly based on morphological similarities, but recent studies integrating comprehensive phenotypic and phylogenetic data have redefined taxonomic limits of several taxa. Here, we assess generic relationships of *Herpsilochmus*, *Sakesphorus*, *Thamnophilus*, *Biatus*, and *Dysithamnus* using DNA sequences from the mitochondrion, nuclear exons, and ultraconserved elements, with further attention to interspecific relationships within *Herpsilochmus*. We show that *Herpsilochmus* and *Sakesphorus* are not monophyletic. We resolve *Herpsilochmus sellowi* as a deep-branch sister to the monotypic genus *Biatus* and *Sakesphorus cristatus* as sister to a clade comprising *Herpsilochmus* sensu stricto and *Dysithamnus*. These results are consistent across loci, obtained via concatenation and coalescent-based analyses, and supported by likelihood-ratio tests of the distribution of our sampled coalescent histories. The phenotypic distinctiveness of both *H. sellowi* and *Biatus* argues against merging them into a single genus. Because no generic name is available for *H. sellowi*, we describe a monotypic genus. The polyphyly of *Sakesphorus* warrants recognition of the available generic name *Sakesphoroides* for the distinctive and monotypic *S. cristatus*. Furthermore, we recover 6 well-supported species groups within *Herpsilochmus* sensu stricto. Within the context of the family as a whole, the ubiquity of long terminal branches representing monotypic genera points to extinction events among ancestors of these lineages. We suggest that retention of ancestral characters or random genetic drift coupled with extensive extinction could explain the high degree of morphological and ecological similarity across these taxa, but we highlight the potential role of the environment in driving adaptive phenotypic convergence. Finally, our results send a cautionary message against the blind use of phylogenies containing imputed data based on taxonomy due to the increasingly frequent mismatches between traditional taxonomic classification and molecular phylogenies.

**Keywords:** antbird phylogeny, monotypic genera, phenotypic constraints, phenotypic convergence, systematics
LAY SUMMARY

- We conduct phylogenomic analyses to infer generic relationships of *Herpsilochmus*, *Sakesphorus*, *Thamnophilus*, *Biatus*, and *Dysithamnus*.
- Using thousands of ultraconserved elements, exons, and mitochondrial DNA, we consistently show that the genus *Herpsilochmus* is not monophyletic because *H. sellowi* is a sister of the monotypic species *Biatus nigropectus*.
- Because the phenotypic distinctiveness of *H. sellowi* and *B. nigropectus* argues against merging them into a single genus and there is no available generic name for *H. sellowi*, we describe a monotypic genus for this species.
- We also found polyphyly of the genus *Sakesphorus*, warranting recognition of the available generic name *Sakesphoroides* for *Sakesphorus cristatus*.
- Our results provide a robust framework for downstream analyses of biogeographic and phenotypic evolution of *Herpsilochmus* antwrens and allies.
- This study adds to the increasing body of literature documenting the mismatch between traditional avian taxonomic classifications based on external morphology and evolutionary histories traced by modern genetic tools.

Análises filogenômicas revelam o não-monofilétismo dos gêneros de papa-formigas *Herpsilochmus* e *Sakesphorus* (Thamnophilidae), com a descrição de um novo gênero para *Herpsilochmus sellowi*

RESUMO

Os representantes da família Thamnophilidae compõem uma radiação Neotropical rica em espécies de passeriformes. A classificação atual das suas 235 espécies é baseada principalmente nas similaridades morfológicas, mas estudos recentes integrando grandes conjuntos de dados fenotípicos e filogenéticos têm redefinido os limites de vários táxons. Nós avaliamos as relações entre os representantes correntemente pertencentes aos gêneros *Herpsilochmus*, *Sakesphorus*, *Thamnophilus*, *Biatus* e *Dysithamnus* usando sequências de DNA mitocondrial, exons nucleares e elementos ultraconservados (UCEs), com atenção adicional às relações interesspecíficas dentro de *Herpsilochmus*. Demonstramos que *Herpsilochmus* e *Sakesphorus* não são monofiléticos, e determinamos que *H. sellowi* é uma espécie-irmã de um ramo profundo do gênero monofilético *Biatus*, enquanto *S. cristatus* é irmã de um clado abrangendo *Herpsilochmus sensu stricto* e *Dysithamnus*. Estes resultados são consistentes através dos diferentes tipos de loci, obtidos via concatenação e análises baseadas em coalescência, e apoiados por testes de razão de verossimilhanças da distribuição das histórias coalescentes amostradas. A distinção fenotípica de *H. sellowi* e *Biatus* argumenta contra a junção deles em um único gênero. Como não há nenhum nome genérico disponível para *H. sellowi*, nós descrevemos um gênero monofilético para esta espécie. A polifilia de *Sakesphorus* garante o reconhecimento do nome genérico disponível *Sakesphoroides* para o distinto e monofilético *S. cristatus*. Além disso, nós recuperamos seis grupos de espécies bem apoiados dentro de *Herpsilochmus sensu stricto*. Dentro do contexto da família como um todo, a ubiquidade dos longos ramos terminais representando os gêneros monofiléticos aponta para eventos de extinção entre os ancestrais dessas linhagens. Nós sugerimos que a retenção de caracteres ancestrais ou deriva genética aleatória, junto a uma extensa extinção, podem explicar o alto grau de similaridade morfológica e ecológica através desses táxons, mas nós destacamos o papel potencial do meio ambiente na condução da convergência fenotípica adaptativa. Por fim, nossos resultados advertem contra o uso cego de filogenias contendo dados baseados na taxonomia, devido às incompatibilidades e entre a classificação taxonômica tradicional e as filogenias moleculares.

Palavras-chave: Novo gênero, gêneros monofiléticos, filogenia dos papa-formigas, convergência fenotípica, restrições fenotípicas, sistemática

INTRODUCTION

The Thamnophilidae is a diverse radiation comprising 235 species of insectivorous passerine birds restricted to the Neotropics (Zimmer and Isler 2003, Dickinson and Christidis 2014, Winkler et al. 2020). They primarily inhabit lower montane and lowland tropical forests and reach their highest diversity in Amazonia, where as many as 50 species may be found at the same locality (Terborgh et al. 1990, Blake 2007, Lees et al. 2013). Co-occurring antbirds tend to vary substantially in body size, plumage, social system, microhabitat use, and behavior, including extreme cases of ecological specialization, such as arboreal dead-leaf searching and army-ant following foraging behaviors (Pearson 1977, Willis and Oniki 1978, Schulenberg 1983, Remsen and Parker 1984, Rosenberg 1993, Robinson and Terborgh 1995, Willson 2004). A likely explanation for such levels of co-existence is the tight association between phenotypic traits and different environmental conditions (Seddon 2005, Bravo et al. 2014, Marcondes and Brumfield 2019, R. Beco et al. personal communication) that, in some cases, has led to convergent evolution across distant relatives and to divergent phenotypes in syntopic species (Tobias and Seddon 2009, Tobias et al. 2010, Bravo

Phylogenetic hypotheses of antbirds based on DNA data mostly assessed generic relationships with incomplete taxonomic sampling (Moyle et al. 2009, Ohlson et al. 2013), and the few instances in which they used species-level data revealed extensive non-monophyly in genera such as Terenura (Bravo et al. 2012b), Myrmeciza (Isler et al. 2013), Myrmotherula (Belmonte-Lopes et al. 2012, Bravo et al. 2012a, 2014), Cercomacra (Tello et al. 2014), Gymnopithys (Isler et al. 2014), and Thamnophilus (Brumfield and Edwards 2007). These studies suggest that non-monophyly might be widespread across currently recognized antbird genera and highlight the necessity to perform explicit tests of phylogenetic relationships in the face of robust taxonomic, genetic, ecological, behavioral, and phenotypic data (Isler et al. 2013). This integrative approach is particularly relevant in species-rich genera with wide distributions across several biomes.

The genus Herpsilochmus currently comprises 17 species (Remsen et al. 2020, Winkler et al. 2020) of small, sexually dichromatic, and phenotypically homogeneous antwrens (7–14 g) that dwell in the middle and upper strata of forest and scrub vegetation, foraging mostly with mixed-species flocks (Whitney and Alvarez 1998). Differences across species are primarily observed in their vocalizations and adult female plumages (Whitney et al. 2000). The genus is widespread, occurring from lower Middle America south through the subtropical and lower tropical zones of the Andes, and throughout the Guianas and Amazonia, inhabiting both the seasonally dry and continuously humid woodland/forest habitats of eastern Brazil, Paraguay, and northern Argentina. Although species of Herpsilochmus are common members of lowland and mid-elevation forest bird communities, it is uncommon to find more than 2 species in sympatry. However, in northeastern Brazil, where 6 species are known to occur (H. sellowi, H. pileatus, H. atricapillus, H. longirostris, H. pectoralis, and H. rufimarginatus), ranges of 3 species overlap in some areas (Whitney et al. 2000, Olmos and Albano 2012, Silva et al. 2012, Ruiz-Esparza et al. 2015, Costa et al. 2016, da Silva et al. 2017), and H. sellowi is sympatric with 4 other species (Whitney et al. 2000).

Taxonomic membership of the genus Herpsilochmus has been universally accepted because these birds form a cohesive morphological, ecological, and behavioral group. However, no formal species-level phylogenetic hypothesis for the genus is available using modern molecular techniques. Current inferences on the phylogenetic position of Herpsilochmus within the Thamnophilidae are based on studies with incomplete taxon sampling and only a handful of markers. They suggest that the genus belongs to the tribe Thamnophilini (sensu Moyle et al. 2009, Ohlson et al. 2013) and that its closest relatives are some species in the genera Dysithamnus, Sakesphorus, and Thamnophilus (Gómez et al. 2010). However, because DNA-based phylogenetic analyses have shown that some of these genera are not monophyletic (Brumfield and Edwards 2007), and some species, such as Sakesphorus cristatus, have been suggested to belong elsewhere in the family (Grantsau 2010), the generic relationships and monophyly of these genera remain in question.

Recent taxonomic changes within Herpsilochmus have resulted from descriptions of new taxa (Davis and O’Neill 1986, Whitney and Alvarez 1998, Cohn-Haft and Bravo 2013, Whitney et al. 2013) and reassessments of species limits (Davis and O’Neill 1986, Whitney et al. 2000). The most prototypically distinct species in the genus is the Brazilian endemic H. sellowi due to its voice—both songs and calls—and remarkably narrow bill, tarsi, and rectrices. Although specimens of H. sellowi had been identified as Herpsilochmus pileatus (Davis and O’Neill 1986 and older literature), Whitney et al. (2000) showed that those specimens represented an unnamed species morphologically and vocally diagnosable from all other members of the genus. In describing H. sellowi as a new species, they regarded it as “a highly distinctive species, both morphologically and vocally” and pointed out that “a reasonable argument could be made for its generic separation.” However, they stopped short of describing a monotypic genus pending phylogenetic evidence for the degree of relationship between H. sellowi and the name-bearing type species of the genus, H. pileatus.

Here, we assess generic relationships of Herpsilochmus, Sakesphorus, Thamnophilus, Biatas, and Dysithamnus, documenting the non-monophyly of Herpsilochmus and Sakesphorus. We corroborate H. sellowi as a highly distinctive taxon that is more closely related to the distinct Biatas nigropectus than it is to other members of the genus Herpsilochmus. Given its distinctive phenotype and phylogenetic position distant to all other members of that otherwise remarkably cohesive Herpsilochmus, we erect a new genus for H. sellowi. We also show that the distinctive and monotypic S. cristatus is not closely related to the other 2 species in the genus, warranting its placement in the available genus Sakesphoroides. Finally, we present a species-level phylogeny of Herpsilochmus that represents a solid framework for future studies of diversification within the genus.
MATERIALS AND METHODS

Taxonomic and Genetic Sampling

To evaluate phylogenetic relationships of *Herpsilochmus*, *Sakesphorus*, *Thamnophilus*, *Biatas*, and *Dysithamnus*, we sampled a total of 115 vouchered specimens of thamnophilids, including all specific and most subspecific taxa within *Herpsilochmus* (we lacked samples of *H. axillaris puncticeps* and *H. roraimae kathleenae*), all species within *Sakesphorus* (*S. canadensis*, *S. luctuosus*, and *S. cristatus*), the type species of *Thamnophilus*, *Biatas*, and *Dysithamnus*, and the outgroups *Formicivora grisea* and *Euchrepomis callinota* (Supplementary Material Table S1). Considering *Herpsilochmus* only, this sampling included an average of 5.1 individuals/species (min = 1; max = 15) and constitutes a comprehensive view of the phylogenetic history of the genus.

We generated 2 separate datasets. The first dataset included a subset of 36 samples (Supplementary Material Table S1) for which we followed a target enrichment approach to sequence ultraconserved elements (UCEs; Faircloth et al. 2012) and nuclear exons across the genome (Zucker et al. 2016). We conducted target enrichment as part of a recent study aimed at reconstructing a species-level phylogenetic history of the suborder Tyranni (Harvey et al. 2020); details on raw read and data processing are published elsewhere (Harvey et al. 2020). Here, we included sequence data for 2 individuals of *H. sellowi*, 22 of other *Herpsilochmus*, 1 of *Biatas*, 2 of *Dysithamnus*, 5 of *Sakesphorus*, 2 of *Thamnophilus*, and 2 outgroups. After quality control, trimming, assembly, and filtering following the Phyluce documentation (Faircloth 2016), we restricted our analyses to those loci containing sequences for at least 30 of the 36 samples in their alignments. Thus, this dataset comprised a total of 1,430,229 base pairs (bp) distributed in 2,142 regions flanking UCEs (1,415,745 bp; an average of 661 bp per locus) and 27 exons (14,484 bp; an average of 536 bp per locus). We provide the alignments used for analyses at https://doi.org/10.5061/dryad.rn8pk0p90 and the raw data are available at NCBI BioProject PRJNA655842 (see Supplementary Material Table S1 for SRA numbers).

The second dataset included sequences for a total of 103 individuals of the complete mitochondrial gene NADH dehydrogenase 2 (ND2, 1,041 bp). We have published some of these sequences elsewhere (Brumfield et al. 2007, Brumfield and Edwards 2007, Gómez et al. 2010, Bravo et al. 2012b, 2014, Cohn-Haft and Bravo 2013, Whitney et al. 2013) and we obtained the rest as part of this project (see Supplementary Material Table S1 for GenBank accession numbers). In total, this dataset included ND2 sequences for 7 individuals of *H. sellowi*, 85 of other *Herpsilochmus*, 2 of *Biatas*, 4 of *Dysithamnus*, 8 of *Sakesphorus*, 3 of *Thamnophilus*, and 2 outgroups.

Total DNA was extracted from 25 mg of pectoral muscle using the Qiagen DNeasy kit following the manufacturer’s protocol, and polymerase chain reactions (PCRs) used the protocols of Brumfield et al. (2007). For one of the samples, *Herpsilochmus axillaris senex* (ANSP 158009), we obtained DNA directly from the specimen’s toepads by allowing a longer digestion time in the presence of dithiothreitol in a facility dedicated to work with ancient DNA to avoid contamination. Clean ND2 PCR products were produced at the Louisiana State University Museum of Natural History (LSUMNS), Laboratório de Biologia Molecular do Museu de Zoologia da Universidade de São Paulo, and Laboratório de Biologia Molecular do Departamento de Zoologia da Universidade Federal do Paraná, and they were bidirectionally sequenced to verify accuracy at LSUMNS, the Louisiana State University Genomics Facility. For library preparation and sequence capture following the general protocol described by Faircloth et al. (2012), we quantified concentrations of DNA extracts using a Qubit fluorometer (ThermoFisher, Waltham, Massachusetts, USA) and sent at least a total of 1 μg of DNA per sample to Rapid Genomics (Gainesville, Florida, USA) for targeted enrichment and Illumina paired-end sequencing.

Phylogenetic Analyses

Gene tree estimation. Unrooted gene trees for UCEs (2,142; 36 individuals), exons (27; 36 individuals), and ND2 (103 individuals) were inferred in the program IQ-TREE 1.6.10 (Nguyen et al. 2014), using the -s, -bb, and -alrt flags to automatically select the best-fit substitution model, and conduct the tree search, SH-aLRT test (Anisimova et al. 2011), and ultrafast bootstrap with 1,000 replicates. For downstream analyses, we used the phylogenetic toolkit phyx (Brown et al. 2017) to root, subsample, and extract bipartition information from gene trees.

Phylogenetic inference. We estimated the phylogenetic hypothesis for the genera *Herpsilochmus*, *Sakesphorus*, *Thamnophilus*, *Biatas*, and *Dysithamnus* using the UCE and exon data (2,169 loci). First, using the software ASTRAL III 5.7.3 (Zhang et al. 2018), we inferred a gene-tree-based coalescent species tree using the unrooted gene trees inferred above and assessed node support via local posterior probabilities estimated based on gene tree quartet frequencies (Sayyari and Mirarab 2016). For this analysis, to avoid potential biases resulting from missing data (Xi et al. 2015a), we removed all sequences of *H. axillaris senex* ANSP 158009 because its representation across loci was low relative to other samples (1,516 loci). Second, including all samples, we conducted a concatenated analysis partitioned by locus using the GTRCAT substitution model and 100 bootstraps in the software RAxML 8 (Stamatakis 2014) implemented on the Cipres Science Gateway 3.3 (Miller et al. 2010).
Coalescent-based tests of alternative genus-level topologies. Guided by phylogenetic inference results (see details below), we used a reduced subset that included no missing data for the highest quality sample of each genus (1,683 loci for all of the 9 individuals; Supplementary Material Table S1), to test how different alternative topologies conformed to the variation of gene tree histories in our dataset. The 4 topologies that were tested considered a *H. sellowi*—*Biatas* or a *H. sellowi*—*Herpsilochmus* sister relationship and 2 alternative placements of *S. cristatus* yielded by the species tree and concatenated analyses (see details below). Using the software MP-EST (Liu et al. 2010) implemented in the R 3.6.2 (R Core Team 2017) package *phybase* 2.0 (Liu and Yu 2010), we first estimated the likelihood of each of the alternative topologies. Then, using the function *test2sptree* in *phybase*, we compared topologies differing only in the placement of *H. sellowi* while maintaining the position of *S. cristatus*. Therefore, the placement of *H. sellowi* was assessed twice: once considering *S. cristatus* as sister to a clade containing *Biatas*, *Dysithamnus*, *H. sellowi*, and *Herpsilochmus*, and a second time considering it sister only to *Dysithamnus* and *Herpsilochmus*. Finally, we compared the most likely topologies resulting from the 2 assessments, yielding the overall topology that best fits the estimated gene tree distribution. These comparisons were conducted using the likelihood-ratio test described by Liu et al. (2019), in which the log-likelihood of 2 alternative topologies is compared with the log-likelihood under the topology of tree 1, which creates a null distribution of *t*. 

\[
t = 2(\text{log-lik}_{\text{tree}2} - \text{log-lik}_{\text{tree}1})
\]

The null distribution of *t* is generated by bootstrapping gene trees and estimating their log-likelihood under the topology of tree 1, which creates a null distribution of *t*. If the observed value of *t* is outside the null distribution of *t*, one can conclude that tree 2 is a better fit to gene tree than tree 1. We ran our comparisons using 999 bootstrap replicates.

**Morphological Variation of Herpsilochmus and Closely Related Genera**

Beyond the key to identification of species in the *H. pileatus* complex and *H. sellowi* presented by Whitney et al. (2000), we used morphometric data to assess the diagnosability of *H. sellowi* as a distinct genus with respect to its closest relatives. We compiled weight data and measured 10 morphometric variables (wing length, primary 10 length, tail length, rectrix 1 width, secondary 1 length, bill length from nostril to tip, bill width at nostrils, bill depth at nostrils, tarsus length, and hallux length) from 242 individuals (see raw data in Supplementary Material Table S2 or at https://doi.org/10.5061/dryad.rn8pk0p90) in the genera *Herpsilochmus*, *Dysithamnus*, *Thamnophilus*, *Sakesphorus*, and *Biatas*. Weight was obtained from specimen labels when available and morphometric measurements were taken to the nearest 0.01 mm with a Mitutoyo Digimatic Point Caliper by GAB. Details of how these measurements were obtained can be found elsewhere (Baldwin et al. 1931, Derryberry et al. 2011). To assess diagnosability among those clades recovered in our phylogenomic analyses representing genera (see details below), we compared mean values of morphometric variation among clades and conducted discriminant function analyses (DFAs) using ln-transformed measurements of morphometric traits for all individuals implemented with the *Ida* function of the R package MASS 7.3-51.6 (Venables and Ripley 2002).

**RESULTS**

Gene-tree-based coalescent and concatenated analyses of 2,142 UCEs and 27 nuclear exons produced almost identical and well-supported results suggesting that *H. sellowi* is a highly distinctive taxon that falls outside the genus *Herpsilochmus* (Figure 1). This result was also strongly supported in the mtDNA analysis with extended intraspecific sampling (Figure 2). The only topological difference across analyses was the position of *S. cristatus*. The concatenated analysis recovered *S. cristatus* as sister to a clade containing *Biatas*, *H. sellowi*, *Dysithamnus*, and *Herpsilochmus*, whereas the gene-tree-based coalescent species tree analysis placed it sister to a clade formed by *Dysithamnus* and *Herpsilochmus*. The mitochondrial gene tree was congruent with the species tree topology in recovering *S. cristatus* as a sister of *Dysithamnus* and *Herpsilochmus* but lacked statistical support. No analysis recovered *S. cristatus* within the clade of the 2 other species of *Sakesphorus* (Figures 1 and 2).

Coalescent-based tests of alternative genus-level species tree topologies revealed that the best-fit species tree, given the distribution of the coalescent histories of a subset of 1,683 loci, was the one uncovered initially by our gene-tree-based coalescent species tree analysis (Figure 3). In other words, these analyses confirmed that *H. sellowi* is not sister to any other species in the genus *Herpsilochmus*, including the type species—*H. pileatus*—and that its sister taxon is the monotypic *B. nigropectus*. Also, coalescent-based tests corroborated *S. cristatus* as sister to a clade formed by the genera *Dysithamnus* and *Herpsilochmus* sensu stricto (Figure 3). Out of the total 247 bipartitions found across the 1,683 genes trees, the one formed by *H. sellowi* and *Biatas* was the most common bipartition splitting 2 genera from the rest and the third most frequent overall; it was observed in 446 gene trees, which were 26.5% of gene trees (Figure 3; Supplementary Material Table S3). The most frequent bipartitions were those at the base of the family (100% of gene trees) and at the base of the tribe Thamnophilini (48.2%). Across gene trees, all other sister relationships for *H. sellowi* were observed 4 times less frequently than those with *Biatas* (Figure 3).

Within the context of the tribe Thamnophilini (sensu Moyle et al. 2009), the divergence between *H. sellowi* and...
Non-monophyly of *Herpsilochmus* and *Sakesphorus*


**FIGURE 1.** Phylogenetic relationships of *Herpsilochmus*, *Sakesphorus*, *Thamnophilus*, *Biatus*, and *Dysithamnus* inferred using gene-tree-based coalescent (left) and partitioned concatenated maximum-likelihood analyses (right) of 2,142 UCEs and 27 exons in the nuclear genome obtained from 36 individuals (Supplementary Material Table S1). Both analyses are congruent in revealing that *Herpsilochmus sellowi* is not closely related to other members of the genus *Herpsilochmus*, including its type species *H. pileatus*. The only topological difference between these analyses was the position of *Sakesphorus cristatus* (blue node on the concatenated tree), which, regardless of the analysis, is not closely related to other *Sakesphorus*, including its type species *S. canadensis*. Numbers at nodes of the gene-tree-based coalescent tree denote local posterior probabilities (Sayyari and Mirarab 2016). Numbers at nodes of the concatenated tree indicate bootstrap support values based on 100 maximum-likelihood replicates. Bird illustrations were made and provided with permission by A. Silva.
FIGURE 2. Maximum-likelihood gene tree of the mitochondrial gene ND2 (1,041 bp) including 101 individuals showing congruent results with UCE-based analyses regarding the non-monophyly of the genera *Herpsilochmus* and *Sakesphorus*. This analysis, as well as those based on UCEs (Figure 1), reveals 6 species groups within *Herpsilochmus* sensu stricto. Numbers at nodes are SH-aLRT support (%) / ultrafast bootstrap support (%). Maps represent the known species geographic distributions (adapted from BirdLife International).
FIGURE 3. Alternative species-tree topologies and their respective log-likelihoods (A–D) were tested using the likelihood-ratio test described by Liu et al. (2019) using 1,683 gene trees that included no missing data. This test compares the log-likelihood of 2 alternative species tree topologies by parametric bootstrapping of the \( t \) statistic, wherein \( t = 2(\text{log-likelihood}_2 - \text{log-likelihood}_1) \). Inset figures show the observed value of \( t \) (red lines) relative to the null distribution of bootstrapped \( t \) value. Comparisons appear as follows: A vs. B: Position of \( H. \) sellowi (A: as sister of \( Herpsilochmus \) vs. B: as sister of \( Biatas \)) when considering \( S. \) cristatus as sister of \( Biatas, Dysithamnus, Herpsilochmus, H. \) sellowi. C vs. D: Position of \( H. \) sellowi (A: as sister of \( Herpsilochmus \) vs. B: as sister of \( Biatas \)) when considering \( S. \) cristatus as sister of \( Dysithamnus \) and \( Herpsilochmus \). B vs. D: Comparison of the favored topologies in previous comparisons. Panel E shows the favored topology (D) with local posterior probabilities from the gene-tree-based species tree (left) and the number of gene trees (1,683 total) that contained that bipartition. Panel F shows the number of gene trees that favor a sister relationship between \( H. \) sellowi and all sampled taxa. Overall, these tests reveal that given the coalescent histories of 1,683 loci, the most likely topology is one describing a sister relationship between \( Biatas \) and \( H. \) sellowi and \( S. \) cristatus as sister \( Dysithamnus \) and \( Herpsilochmus \). Bird illustrations were made and provided with permission by A. Silva.
the monotypic genus *Biatas* is one of the highest observed between 2 sister species (Harvey et al. 2020) and that is evidenced in our analyses by the long internal branch lengths leading to these species. *H. sellowi* appears to exhibit significant genetic divergence within its range, but the extent to which such breaks may conform to discrete

FIGURE 4. Mapping of morphological character states onto the favored topology underlying the relationships of *Herpsilochmus* and their close relatives (A). Discriminant factors of morphometric variation (B). Negative values of Factor 1 represent predominantly deeper and less pointy bills, whereas positive values represent predominantly shallower and pointier bills. Negative values of Factor 2 represent mostly longer wings, whereas positive values represent longer tails. Ellipses represent 95% confidence intervals. Bird illustrations were made and provided with permission by A. Silva.
geographic distributions is currently under study (Figure 2). Aside from our large-scale phylogenomic analyses of suboscines (Harvey et al. 2020), no previous studies suggested a sister relationship between H. sellowi—or any Herpsilochmus—and Biatus, and to combine these 2 taxa in a single genus would disrupt any sense of morphological continuity in thamnophilid genera. Moreover, a DFA of 10 ln-transformed morphometric variables showed that H. sellowi is fully diagnosable from all closely related genera (Wilks’ lambda = 0.022, F_{6,235} = 21.209, P < 2.2E-16; Figure 4). All individuals of H. sellowi, Biatus, Herpsilochmus sensu stricto, and S. cristatus were correctly assigned to their separate group, whereas correct classification percentages were lower for Thamnophilus (98.3%), Sakesphorus sensu stricto (50%), and Dysithamnus (74.2%; Figure 4). Given H. sellowi’s highly distinctive and diagnosable phenotype and phylogenetic position distant to H. pileatus and all other members of that genus, we introduce a new genus for “Herpsilochmus” sellowi, as follows:

Radinopsyche gen. nov. Whitney, Bravo, Belmonte-Lopes, Bornschein, Pie, and Brumfield

urn:lsid:zoobank.org:act:FA02E9C5-8958-4D65-A270-79E51F084D1E

Type species

Herpsilochmus sellowi Whitney and Pacheco 2000

Included species

Radinopsyche sellowi comb. nov., Caatinga Antwren.

Diagnosis

Morphology. The only species belonging to Radinopsyche is significantly smaller, lighter, and slenderer than any of its close relatives. Absolute measurements of its bill, wings, tail, tarsi, and halluces are lower than those of closely related genera, with a few exceptions when compared to Dysithamnus and Herpsilochmus (Figure 4; Supplementary Material Table S4): the former has a tail length similar to Radinopsyche, and the latter has similar tarsus and tail length. Radinopsyche is, however, readily diagnosable from these species by its proportionately longer and slenderer bill and tail. Unlike all members of Herpsilochmus, males of Radinopsyche lack a dark preocular line/spot. Females lack discrete pale markings on the crown (these being replaced by a dull, mottled pattern formed by buff feather edges), which sets it apart from all Herpsilochmus having pale supercilia. Radinopsyche is strikingly different from B. nigropectus, its closest extant relative, in all respects. The latter is a great deal larger (29 g vs. 7 g), crested, and much more strongly sexually dichromatic. It lacks any white or pale spotting on the wings and tail, and it also lacks a dark eyeliner or pale supercilium (except for weak presence in females). Whitney et al. (2000) provided diagnostic morphometrics and a simple diagnostic key for separation of specimens of R. sellowi from the morphologically most similar, sympatric species of Herpsilochmus (H. pileatus and H. atricapillus) with which it had been confused.

Voice. The loudsong of Radinopsyche is immediately diagnosable from all members of Herpsilochmus by its uniformly rapid pace (x = 56 notes in 2.2 s, n = 32 loudsongs) without change in the duration of individual notes or tonality. Some calls are also highly distinctive, perhaps most notably the rattle (all above information from Whitney et al. 2000). The remarkably different loudsong of B. nigropectus may be readily compared by listening to the taxon-representative recordings presented by Isler and Whitney (2002).

Etymology. Radinopsyche is a combination of Greek words to render “slender/gracile being/spirit,” in recognition of the multiple structural characteristics that distinguish sellowi from other, superficially similar but more heavily built antwrens (see Diagnosis: Morphology). The loudsong is also a remarkably thin, airy trill. The new name is neutral in gender.

Another interesting result was the non-monophyly of Sakesphorus. Our results showed that S. cristatus is the most phenotypically distinct species of the genus (Figure 4) and is sister to a clade formed by Herpsilochmus sensu stricto and Dysithamnus (Figure 3). This corroborates previous assertions about the phenotypic distinctiveness of this species (Grantsau 2010) that led to the erection of the genus Sakesphoroides Grantsau 2010. Given the tree topology and observed phenotypic variation across genera, here, we endorse the placement of S. cristatus in Sakesphoroides.

Within the genus Herpsilochmus, we found that H. pectoralis, a species restricted to northeastern Brazil, is sister to all other members of the genus (Figure 1) and henceforth refer to it as the Pectoralis group. The remainder of the genus was organized into 5 distinct clades. The first clade is formed by the widespread H. rufimarginatus (Rufous-winged clade), wherein significant genetic divergence separates the northern subspecies frater and exiguus from the nominate and scapularis, supporting the split of this species into H. frater and H. rufimarginatus. The second clade comprises only the Tepui-restricted H. roraimae (Roraima clade), which, in turn, is sister to the remaining clades in the genus. The third clade includes species restricted to the Andes (H. axillaris), the South American Dry Diagonal (H. longirostris), northwestern Amazonia (H. dugandi), and the Guiana Shield (H. sticturus). We refer to this biogeographically diverse group by the name of its first-described member, the Axillaris clade. The fourth clade includes species from
the Guianan Shield (H. stictocephalus) across northern Amazonia (H. dorsimaculatus), and, more locally, in northwestern Amazonia (H. gentryi). We refer to this group as the Spot-backed clade. Finally, the fifth clade corresponds to the “pileatus complex” as defined by Whitney et al. (2000), which includes species occupying south-central Amazonia (H. stotzi and H. praedictus), the southern Andes (H. mottacillioides and H. parkeri), the South American Arid Diagonal (H. atricapillus), and the coastal Atlantic Forest of Bahia, Brazil (H. pileatus). We refer to it as the Pileatus clade, in recognition of the oldest name in the group, and to help retain consistency in the literature.

DISCUSSION

We show that, as currently recognized, the genus Herpsilochmus is not monophyletic and that H. sellowi should occupy its own genus. Because this species is sister to the highly distinct genus Biatas, we place it in the newly erected monotypic genus Radinopsycha. Moreover, we show that Herpsilochmus is sister to Dysithamnus and that these 2 are in turn sister to S. cristatus. Therefore, we confirm the placement of S. cristatus in the available genus Sakesphoroides. Finally, we present the first species-level phylogeny of the genus Herpsilochmus, which will provide a robust framework for downstream analyses of biogeographic and phenotypic evolution in this group of antwrens.

A distant relationship between R. sellowi and all other members of Herpsilochmus was not unexpected. At the time of its description, Whitney et al. (2000) recognized that R. sellowi fell outside the morphological parameters defining Herpsilochmus identified by Whitney and Alvarez (1998), and its voice—both songs and calls—was remarkably different from that of any member of the genus. Furthermore, in recognition of the fact that R. sellowi is syntopic or sympatric with at least 4 species of Herpsilochmus (H. pectoralis, H. rufimarginatus, H. axillaris, H. pileatus) and no other Herpsilochmus is known to be syntopic with more than 2 congeners (B. M. Whitney personal observation), Whitney et al. (2000) proposed that H. sellowi might merit a separate genus.

Nonetheless, the sister relationship between Radinopsycha and Biatas was a result we never would have imagined, even given our thorough familiarity with both taxa and almost all other thamnophilids in life. Phenotypically (morphology and voice) and ecologically, these are remarkably divergent taxa. Radinopsycha is conspicuously more similar to Sakesphoroides + Herpsilochmus + Dysithamnus, and Biatas is phenotypically and ecologically much more similar to Sakesphoroides + Thamnophilus (Figure 4). Several attributes of our phylogenetic results, such as long internal branches and the high nodal support of the clade comprising Radinopsycha and Biatas, and the high congruence across methods and marker types, suggest that this relationship is not artifactual or biased by pervasive incomplete lineage sorting, recombination, introgression, missing data, or widespread gene tree estimation errors (Degnan and Rosenberg 2006, 2009, Tonini et al. 2015, Xi et al. 2015a, 2015b, Mirarab et al. 2016, Cloutier et al. 2019). Moreover, the fact that this clade corresponds to the most common bipartition between any 2 genera observed across gene trees is also a good indicator of the robustness of this result.

According to the calibration analyses presented by Harvey et al. (2020), the crown ages of the Thamnophilidae and the tribe Thamnophilini are 19.7 Ma and 12.9 Ma, respectively, and the average generic stem age is 9.5 Ma (3.8–19.1 Ma). When considering the stem age estimates by Harvey et al. (2020) of the long terminal branches leading to the monotypic Biatas (8.4 Ma), Radinopsycha (8.4 Ma), and Sakesphoroides (11.2 Ma) and the observed species richness of genera of similar ages such as Thamnophilus (10.5 Ma, ~30 species), Herpsilochmus (9.6 Ma, ~16 species), Hypocnemis (10 Ma, ~8 species), or Cercomacroides (11.1 Ma, ~5 species), it seems evident that these monotypic lineages might have experienced prevalent extinction events. Also, Harvey et al. (2020) found evidence of historical extinction rates being higher in lineages occurring in areas of low species diversity such as the Caatinga—the stronghold of the distribution of Sakesphoroides and Radinopsycha—and identified this pattern in virtually all Neotropical suboscine families. Thus, we predict that this pattern will become more apparent as more time-calibrated molecular phylogenies become available for other groups. Another possibility is that these putative extinct lineages represent taxa unknown to science that persist in unexplored corners of South America despite nearly 400 years of incessant habitat alteration and reduced chances of survival. Recent discoveries of range-restricted taxa such as Acrobatornis fonsekai (Pacheco et al. 1996), Lipaugus weberi (Cuervo et al. 2001), Myrmoderus eowilsoni (Moncrieff et al. 2018), and the yet-undescribed “San Pedro Tanager” (Lane et al. personal communication), or rediscoveries of long-lost species such as Formicitora erythronotos (Pacheco 1988), Pithys castaneus (Lane et al. 2006), or Clytoctantes alicii (Laverde and Stiles 2007) are good examples of how some of these missing links can still be around.

Retention of ancestral characters or random genetic drift coupled with extensive extinction in the clade, as suggested above, can very well explain the high degree of morphological and ecological similarity between Radinopsycha and Herpsilochmus. However, we should consider that in antbirds environmental and ecological associations can drive adaptive intra- (Isler et al. 2005, Sementili-Cardoso et al. 2017, Marcondes et al. 2020) and
interspecific (Seddon 2005, Bravo et al. 2014, Marcondes and Brumfield 2019, Beco et al. personal communication) phenotypic diversity, resulting in phenotypic convergence, as it is also widespread in a wealth of organisms (Hoekstra et al. 2006, Mahler et al. 2013, McGee and Wainwright 2013). Because phenotypic similarity can result from a variety of evolutionary mechanisms that must be adequately tested (reviewed by Losos 2008, 2011, Crisp and Cook 2012, Mahler et al. 2017), at this point we are unable to provide a thorough explanation for the striking parallels of Radinopsychus and Herpsilochmus. However, another potential explanation that might be relatively easily quantified in the field involves competitive interaction between syntopic, interacting species (Scheffer and van Nes 2006, Tóbias et al. 2014).

In the case of Sakesphoridae cristatus, the role of the environment in driving plumage pattern similarities with other species currently or formerly placed in Sakesphorus (i.e. Sakesphorus canadensis and Thamnophilus bernardi) seems to be relevant. These species occur in some of the most prominent seasonally dry forests of South America (Tumbes, Caatinga, Northern Colombia, and Venezuela), where temperatures can be very high and precipitation very low and seasonal (Gentry 1995, Graham and Dilcher 1995, Pennington et al. 2000). Overall, these species have some of the lighter plumages in the family (Marcondes and Brumfield 2019), fitting predictions of Gloger’s rule (Gloger 1833, Delhey 2017, 2019), which seems to be widely supported in the family (Bravo et al. personal communication). Males of these species even share a rather infrequent plumage pattern, with subtle variations (i.e. black face, crown and bib; whitish belly, sides, and flanks; brownish back; blackish tail), that is unique to species in seasonally dry forests. Interestingly, because they are distantly related and occur allopatrically in different regions (S. canadensis in northern Colombia and Venezuela, albeit extending into the Guiana Shield and Northern Amazonia; T. bernardi in the Tumbesian region of western Peru and Ecuador; and Sakesphoridae cristatus in the Caatinga), this unique plumage pattern evolved convergently, likely with some influence of the similar environmental conditions in these regions. Such a striking similarity in plumage pattern led earlier taxonomists to transfer these taxa from the genus Thamnophilus to Sakesphorus (Cory and Hellmayr 1924); a treatment that persisted over time (Zimmer 1933, Meyer de Schauensee 1950, Peters 1951, Sick 1985, Ridgely and Tudor 1994, Zimmer and Isler 2003) and that only recently was modified (Dickinson and Christidis 2014, Remsen et al. 2020, Winkler et al. 2020) due to the advent of DNA-based phylogenetic evidence (Brumfield and Edwards 2007).

Finally, our results add to the increasing body of literature documenting the mismatch between the traditional taxonomic classification of Neotropical birds based on external morphology and their evolutionary history traced by modern genetic tools. This phenomenon has been particularly widespread in instances of extreme phenotypic similarity wherein high levels of homoplasy led early taxonomists to artificial generic classifications (Amaral et al. 2006, Maurício et al. 2008, Bravo et al. 2012b, Klicka et al. 2014, Carneiro et al. 2019, Settlecowski et al. 2020). Such taxonomic reappraisal has been effectively possible thanks to the integration of comprehensive molecular and phenotypic data with robust analytical methods and highlights the essential role of museum collections in modern research (Rocha et al. 2014, Funk 2018, Bakker et al. 2020). Furthermore, the common finding of erroneous taxonomic classifications sends a cautionary message against the blind use of phylogenies containing imputed data based on traditional taxonomic classifications. Although factors such as the proportion of imputed taxa and the size of the tree are critical, if imputed taxa are misplaced in the tree, then they could bias downstream comparative analyses (Davies et al. 2012, Rabosky 2015) and the magnitude of those biases remains mostly unknown (Pennell et al. 2016).

SUPPLEMENTARY MATERIAL

Supplementary material is available at Ornithology online.

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**Ethics statement:** This project primarily used samples from existing genomic resource collections at natural history museums. Therefore, it was exempted by the Louisiana State University Institutional Animal Care and Use Committee (IACUC). Samples from Brazil were collected under Brazilian government SISBio permits 19792-2, 19792-3, 26227-1 (to R.B.-L.), 36496 (to L.N.N.), and 10013-2 (to L.F.S.) following the regulations provided by Brazil’s National Council for the Control of Animal Experimentation (CONCEA).


**ZooBank LSID:** urn:lsid:zoobank.org:pub:0E97169B-C3D3D-4501-8B2B-681A1CAF3FF37

**Data availability:** Raw UCEs and exon data originally published by Harvey et al. (2020) are available at NCBI BioProject PRJNA655842 (see Supplementary Material Table S1 for SRA numbers). Newly generated ND2 sequences are available at GeneBank (accession numbers MW713856–MW713916; see Supplementary Material Table S1 for detailed information). Alignments, tree files, and raw morphometric data can be found at Bravo et al. (2021).

**LITERATURE CITED**


