

MOLECULAR SYSTEMATICS AND PLUMAGE EVOLUTION IN THE MONOTYPIC OBLIGATE ARMY-ANT-FOLLOWING GENUS *SKUTCHIA* (THAMNOPHILIDAE)

ALEXANDRE ALEIXO^{1,3}, TIBÉRIO C. T. BURLAMAQUI², M. PAULA C. SCHNEIDER²,
 AND EVONNILDO C. GONÇALVES²

¹Coordenação de Zoologia, Museu Paraense Emílio Goeldi, Caixa Postal 399, CEP 66040-170, Belém, Pará, Brazil

²Laboratório de Polimorfismo de DNA, DEGEN, Universidade Federal do Pará, Belém, Pará, Brazil

Abstract. We obtained 1403 base pairs of mtDNA sequences of *cyt b* and ND2 from four specimens of the Pale-faced Bare-eye (*Skutchia borbae*, representing a monotypic genus of the Thamnophilidae) and evaluated for the first time the species' phylogenetic position among core obligate army-ant-following antbirds. All trees placed *Skutchia borbae* consistently and with high statistical support as sister to the Black-spotted Bare-eye (*Phlegopsis nigromaculata*) in a clade with the two known species of *Phlegopsis*. This result supports the return of *S. borbae* to the genus *Phlegopsis*, where it was originally placed. When the plumage characters originally used to diagnose *Skutchia* are mapped onto the phylogeny, it is evident that they have evolved convergently multiple times in core obligate army-ant-following antbirds. This discovery further supports merging *Skutchia* with *Phlegopsis*.

Key words: *Molecular systematics, Phlegopsis, plumage evolution, Skutchia borbae, taxonomy, Thamnophilidae.*

Sistemática Molecular y Evolución del Plumaje en *Skutchia*, un Género Monotípico que Sigue Ejércitos de Hormigas de Modo Obligatorio (Thamnophilidae)

Resumen. Obtuvimos 1403 pares de bases de secuencias de ADNmt (*cyt b* y ND2) de cuatro especímenes de *Skutchia borbae*, un género monotípico de la familia Thamnophilidae, y evaluamos por primera vez su posición filogenética entre las especies de Thamnophilidae que siguen ejércitos de hormigas obligatoriamente. Todos los árboles ubicaron a *S. borbae* consistentemente y con alto respaldo estadístico como especie hermana de *Phlegopsis nigromaculata* en el clado que incluye a las dos especies conocidas de *Phlegopsis*. Este resultado apoya el retorno de *S. borbae* al género *Phlegopsis*, donde había sido ubicada originalmente. Al mapear en la filogenia los caracteres del plumaje que fueron originalmente utilizados para diagnosticar a *Skutchia*, es evidente de que éstos han evolucionado varias veces de forma convergente en los seguidores de hormigas obligados. Este hallazgo da aún más apoyo para combinar los géneros *Skutchia* y *Phlegopsis*.

The monotypic genus *Skutchia* was erected by Willis (1968) for the species originally described as *Phlegopsis borbae* (Pale-faced Bare-eye) on the basis of a single specimen from Borba, on the east (right) bank of the lower Madeira River (Hellmayr 1907). The species' range is bounded by the Madeira and Tapajós rivers in the Brazilian Amazon. Until Willis (1968), knowledge of *P. borbae* was restricted to two specimens (including the holotype) obtained from what appeared to be the margins of its distribution. Willis (1968) reported extensive data on behavior and ecology of *P. borbae* and demonstrated that it belongs to the guild of obligate army-ant-following birds along with *Phlegopsis* and several other genera. He argued that *P. borbae* was morphologically so distinct from any other army-ant-following species that it should be treated as separate genus, which he named *Skutchia* in honor of Alexander Skutch, the famous neotropical ornithologist.

In the original diagnosis, Willis (1968) *Skutchia* was distinguished from *Phlegopsis* by (1) the reduced extent and paler color of the bare skin in its orbital area, (2) its patch of black stiffened feathers above the eye, (3) the distinct patch of elongated feathers on the lores, (4) the body coloration in which adult males are predominantly brownish rather than blackish, and (5) its distinct pectoral band. Since the description of *Skutchia*, the placement of *P. borbae* in this genus has been followed by most authors (Zimmer and Isler 2003, Brumfield et al. 2007, Remsen et al. 2009), even though the consistency of the diagnosis with respect to *Phlegopsis* has been questioned at least once (Pinto 1978).

Brumfield et al. (2007) proposed the first phylogenetic hypothesis for the army-ant-following antbirds (Thamnophilidae), but for lack of tissues, *Skutchia* was the only obligate army-ant follower genus they did not analyze (R. Brumfield, pers. comm.). Nonetheless, from its taxonomic history, Brumfield et al. (2007) argued that *Skutchia* is likely positioned within *Phlegopsis*.

Here, we analyze 1403 bp of cytochrome *b* (*cyt b*) and NADH dehydrogenase 2 (ND2) mtDNA sequences from four specimens of *S. borbae* in association with the data presented by Brumfield et al. (2007) to provide the first phylogenetic analysis that includes *Skutchia*. The analysis demonstrates not only the phylogenetic position of *Skutchia* but also evaluates the plumage characters used to diagnose the genus.

METHODS

In addition to *Skutchia*, we sampled all core obligate army-ant-following genera of the Thamnophilidae (*Pithys*, *Gymnopithys*,

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³E-mail: aleixo@museu-goeldi.br

TABLE 1. General information on the samples of *Skutchia borbae*, *Phlegopsis erythroptera*, and *Phlegopsis nigromaculata* sequenced in this study. All samples were ethanol-preserved muscle tissues. Voucher numbers refer to study skins deposited at the ornithological collection of the Museu Paraense Emílio Goeldi in Belém, Brazil (MPEG).

Taxon	Voucher number	Locality	Cyt <i>b</i> ^a	ND2 ^a
<i>S. borbae</i>	MPEG 57691	Brazil: Amazonas; Manicoré, Rodovia do Estanho, km 136 (8° 41' S, 61° 24' W)	FJ804573	FJ804584
<i>S. borbae</i>	MPEG 57693	Brazil: Amazonas; Manicoré, Rodovia do Estanho, km 136 (8° 41' S, 61° 24' W)	FJ804574	FJ804585
<i>S. borbae</i>	MPEG 57694	Brazil: Amazonas; Manicoré, Rodovia do Estanho, km 136 (8° 41' S, 61° 24' W)	FJ804575	FJ804586
<i>S. borbae</i>	MPEG 58755	Brazil: Amazonas; Humaitá, Terra Indígena Parintintin, Aldeia Traira-Chororó (7° 33' S, 62° 33' W)	FJ804576	FJ804587
<i>P. e. erythroptera</i>	MPEG 59609	Brazil: Amazonas; Novo Airão, Igarapé-Açu (2° 51' S, 60° 51' W)	FJ804577	FJ804588
<i>P. e. ustulata</i>	MPEG 57142	Brazil: Amazonas, Coari, Rio Urucú (4° 51' S, 65° 04' W)	FJ804578	FJ804589
<i>P. n. nigromaculata</i>	MPEG 59868	Brazil: Acre, Assis Brasil, ESEC Rio Acre (11° 03' S, 70° 16' W)	FJ804579	FJ804590
<i>P. n. bowmani</i>	MPEG 58133	Brazil: Pará, Juruti, Omnia Minérios (2° 28' S, 56° 00' W)	FJ804581	FJ804592
<i>P. n. bowmani</i>	MPEG 56103	Brazil: Pará, Belterra, FLONA Tapajós (3° 21' S, 54° 56' W)	FJ804582	FJ804593
<i>P. n. confinis</i>	MPEG 55694	Brazil: Pará, UHE Belo Monte, Senador José Porfírio (3° 31' S, 51° 44' W)	FJ804583	FJ804594
<i>P. n. paraensis</i>	MPEG 58980	Brazil: Pará, Paragominas, Fazenda Rio Capim (3° 42' S, 48° 33' W)	FJ804580	FJ804591

^aGenBank accession numbers.

Rhegmatorhina, *Phlegopsis*, and *Phaenostictus*), shown to be consistently monophyletic (clade A of figure 2 in Brumfield et al. 2007). We obtained sequences for *Skutchia* from ethanol-preserved muscle tissues vouchered by four study skins deposited at the ornithological collection of the Museu Paraense Emílio Goeldi in Belém, Brazil (MPEG) and collected at two localities separated by about 200 km in the southern part of the Brazilian state of Amazonas (Table 1). To ensure dense taxon sampling of *Phlegopsis*, the genus purportedly closest to *Skutchia*, we also sequenced at least one representative of each taxon in this genus recognized by Zimmer and Isler (2003; see Table 1). All remaining sequences used in this study were downloaded from GenBank (Brumfield et al. 2007), representing the White-plumed Antbird (*Pithys albifrons*: EF640047, EF639980), White-masked Antbird (*P. castaneus*: EF640060, EF639993), Bicolored Antbird (*Gymnopithys leucaspis leucaspis*: EF640062, EF639995, and *G. l. bicolor*: EF640061, EF639994), Rufous-throated Antbird (*G. rufigula*: EF640064, EF639997), White-throated Antbird (*G. salvini*: EF640016, EF639949), Lunulated Antbird (*G. lunulatus*: EF640063, EF639996), Bare-eyed Antbird (*Rhegmatorhina gymnops*: EF640065, EF639998), White-breasted Antbird (*R. hoffmannsi*: EF640050, EF639983), Hairy-crested Antbird (*R. melanosticta*: EF640066, EF639999), Scale-backed Antbird (*Willisornis poecilinotus*: EF640020, EF639953), Black-spotted Bare-eye (*Phlegopsis nigromaculata*: EF640046, EF639979), Reddish-winged Bare-eye (*P. erythroptera*: EF640068, EF640001), and Ocellated Antbird (*Phaenostictus mcleannani*: EF640045, EF639978).

For all samples shown in Table 1, we amplified portions of the *cyt b* and ND2 mitochondrial protein-coding genes by PCR under standard conditions (available upon request) with the following primers, respectively: L14990 (Helm-Bychowski and

Cracraft 1993)/H16065 (Kocher et al. 1989) and L5215 (Hackett 1996)/H6313 (Sorenson et al. 1998). PCR products were cycle-sequenced with a DYEnamic ET Dye Terminator kit (GE Healthcare) and run on a DNA MegaBace (GE Healthcare) sequence analyzer according to the manufacturer's instructions. The following measures were taken to ensure that the DNA fragments sequenced were accurate and of mitochondrial origin (not pseudogenes): (1) both DNA strands were sequenced, (2) sequences were inspected for insertions, deletions, and stop codons that would result in a nonfunctional protein with the aid of the software BioEdit (Hall 1999) and DAMBE (Xia and Xie 2001), and (3) sequences were expected to exhibit high transition-to-transversion substitution ratios characteristic of mitochondrial, not nuclear, substitution patterns.

Phylogenetic analyses were performed on the concatenated *cyt b*/ND2 dataset, after a partition-homogeneity test as implemented in PAUP* 4.0b10 (Swofford 2002) failed to detect significantly different phylogenetic signals between the two genes analyzed ($P > 0.05$). Trees were rooted in *Willisornis poecilinotus* (a regular army-ant follower), shown with strong statistical support to be the sister taxon to the entire core obligate army-ant-following clade (Brumfield et al. 2007).

Maximum-parsimony (MP) and maximum-likelihood (ML) searches were conducted with PAUP* 4.0b10, with MP based on unweighted sequence data and ML following the model of molecular evolution chosen by the Akaike information criterion (AIC) that fit our dataset, as selected by the software Modeltest 3.7 (Posada and Crandall 1998). We used 1000 nonparametric bootstrap replications to evaluate confidence levels of nodes for all phylogenies obtained with MP and ML (Felsenstein 2004). Using the software MrBayes 3.1.2, we also assessed the support of nodes in the phylogeny with a site-partitioned Bayesian analysis

TABLE 2. Distribution of plumage characters used by Willis (1968) to diagnose *Skutchia* among core obligate army-ant-following antbirds.

Taxon	Character number ^a				
	1	2	3	4	5
<i>Pithys albifrons</i>	1	0	0	1	0
<i>Pithys castaneus</i>	1	1	0	0	0
<i>Gymnopithys l. leucaspis</i>	1	1	0	0	0
<i>Gymnopithys l. bicolor</i>	1	1	0	0	0
<i>Gymnopithys rufigula</i>	0	1	0	0	0
<i>Gymnopithys salvini</i>	0	0	0	0	0
<i>Gymnopithys lunulatus</i>	0	0	0	0	0
<i>Rhegmatorhina gymnops</i>	1	1	0	0	0
<i>Rhegmatorhina hoffmannsi</i>	1	1	0	0	1
<i>Rhegmatorhina melanosticta</i>	1	1	0	0	0
<i>Willisornis poicilinotus</i>	0	0	0	0	0
<i>Phlegopsis nigromaculata</i>	1	2	0	0	0
<i>Phlegopsis erythroptera</i>	1	2	0	0	0
<i>Skutchia borbae</i>	0	1	1	1	1
<i>Phaenostictus mcleannani</i>	1	1	1	0	1

^aKey for characters and character states: (1) black in the body coloration of males: 0 = absent, 1 = present; (2) color intensity of the bare orbital skin: 0 = absent, 1 = light, 2 = dark; (3) patch of stiffened black feathers above the eye: 0 = absent, 1 = present; (4) patch of elongated feathers on the lores: 0 = absent, 1 = present; (5) barring on the chest and belly: 0 = absent, 1 = present.

(Ronquist and Huelsenbeck 2003) with the following specifications: (1) assuming separate optimal ML models selected for each data partition by Modeltest 3.7; (2) running the Markov chain for 5 000 000 generations and sampling 1 tree every 1000 generations. We discarded the first 499 trees sampled (10% of the sample) as “burn-in”, with the remaining 4501 trees used to estimate posterior probabilities of the trees’ topologies.

We mapped the plumage characters used by Willis (1968) to diagnose *Skutchia* onto the phylogeny of core obligate army-ant-following antbirds from figure 2 of Brumfield et al. (2007), complemented with our data for *Skutchia borbae* (Table 2). For each terminal taxon in the phylogeny, we examined adult museum specimens at MPEG and used Zimmer and Isler (2003) as a reference to code characters of taxa missing from the collection (*Gymnopithys leucaspis bicolor*, *G. lunulatus*, and *Pithys castaneus*). All characters were scored as unordered and either binary or multistate. We used MACCLADE (version 3.07; Maddison and Maddison 1992) to reconstruct patterns of character evolution and to assess whether characters have significant phylogenetic signal. We calculated the consistency index (CI) and retention index (RI) for each character mapped onto the phylogeny. We used Maddison and Slatkin’s (1991) randomization test to assess the phylogenetic signal or inertia of these characters. For each character, we randomized the character states 1000× on the phylogeny and compared the reconstructed number of character-state changes to the random distribution of character-state changes. Characters were reconstructed by both acctran and deltran optimization.

RESULTS

Cyt *b* and ND2 sequences from 11 individuals of *Skutchia borbae*, *Phlegopsis nigromaculata*, and *P. erythroptera* obtained in

this study (see Table 1) were trimmed to 453 bp and 950 bp, respectively, spanning positions 15307 to 15759 and 5286 to 6235 of the cyt *b* and ND2 sequences of the domestic chicken (*Gallus gallus*) (Desjardins and Morais 1990). Aligned sequences revealed no signs of saturation (plot available upon request) and were deposited in GenBank (see Table 1).

The newly sequenced dataset of 1403 nucleotides of *Skutchia* and *Phlegopsis* was combined with data on *Pithys*, *Phaenostictus*, *Gymnopithys*, *Rhegmatorhina*, *Phlegopsis*, and *Willisornis* downloaded from GenBank (Brumfield et al. 2007). This 25-taxon dataset had 464 variable sites (33%), 347 of which were phylogenetically informative. An equally weighted MP heuristic search of the entire dataset resulted in a single most parsimonious tree (length 949; CI = 0.57; RI = 0.76; not shown). Modeltest analysis selected the TrN + I + G model of nucleotide substitution with estimated base frequencies (A = 0.32, C = 0.34, G = 0.09, T = 0.25), substitution model (A–C = 1, A–G = 22.77, A–T = 1, C–G = 1, C–T = 15.04, G–T = 1), proportion of invariable sites (*I* = 0.60), and rates for variable sites following a gamma distribution (Γ shape parameter = 4.51). The ML tree (–ln likelihood = 6409.32293; Fig. 1) had a topology similar to that estimated by MP, except for the position of the *Pithys/Phaenostictus* clade, which was placed as sister to the *Rhegmatorhina/Gymnopithys* clade in the latter analysis.

Regardless of the tree-building method employed, all phylogeny estimates placed *Skutchia borbae* consistently and with high statistical support in a clade with the two known species of the genus *Phlegopsis*; relationships within this clade were also well resolved and statistically supported and showed *S. borbae* and *P. nigromaculata* as sister taxa, to the exclusion of *P. erythroptera* (Fig. 1). MP and ML estimates differed, respectively, in placing an entire *Pithys/Phaenostictus/Rhegmatorhina/Gymnopithys* clade or just a *Pithys/Phaenostictus* clade as sister to the *Phlegopsis/Skutchia* clade. Irrespective of the tree-building method, a very short branch and poorly supported node joins the *Phlegopsis/Skutchia* clade to its sister group (Fig. 1), reflecting the same lack of resolution concerning the sister taxon of *Phlegopsis* found by Brumfield et al. (2007).

When mapped onto molecular phylogeny (cladograms not shown), none of the five plumage characters used by Willis (1968) to distinguish *Skutchia* from other obligate army-ant-following antbird genera exhibited a significant phylogenetic signal (Table 3). The characters (3) patch of stiffened black feathers above the eye and (4) patch of elongated feathers on the lores represent autapomorphies that have evolved independently in *Skutchia* and *Phaenostictus* and in *Skutchia* and *Pithys*, respectively. Similarly, character 5 (extensive barring on the chest and belly) is an autapomorphy that has evolved independently in *Skutchia*, *Rhegmatorhina*, and *Phaenostictus*. The lack of black plumage in males of *Skutchia* (character 1) is also shared with two independent lineages in the genus *Gymnopithys*, and all have evolved from a common ancestor in which males had black in the plumage. Finally, the light bare orbital skin (character 2) of *Skutchia* represents either a reversal from the dark red shown by *Phlegopsis* (in acctran optimizations) or retention of a character inherited from an ancestor with light bare skin (in deltran optimizations).

DISCUSSION

The phylogenies in Fig. 1 and Brumfield et al. (2007) confirm the long-standing belief based on external morphology, vocalizations, behavior, and ecology that *Skutchia* belongs in the clade of core obligate army-ant-following antbirds (Willis 1968, Zimmer

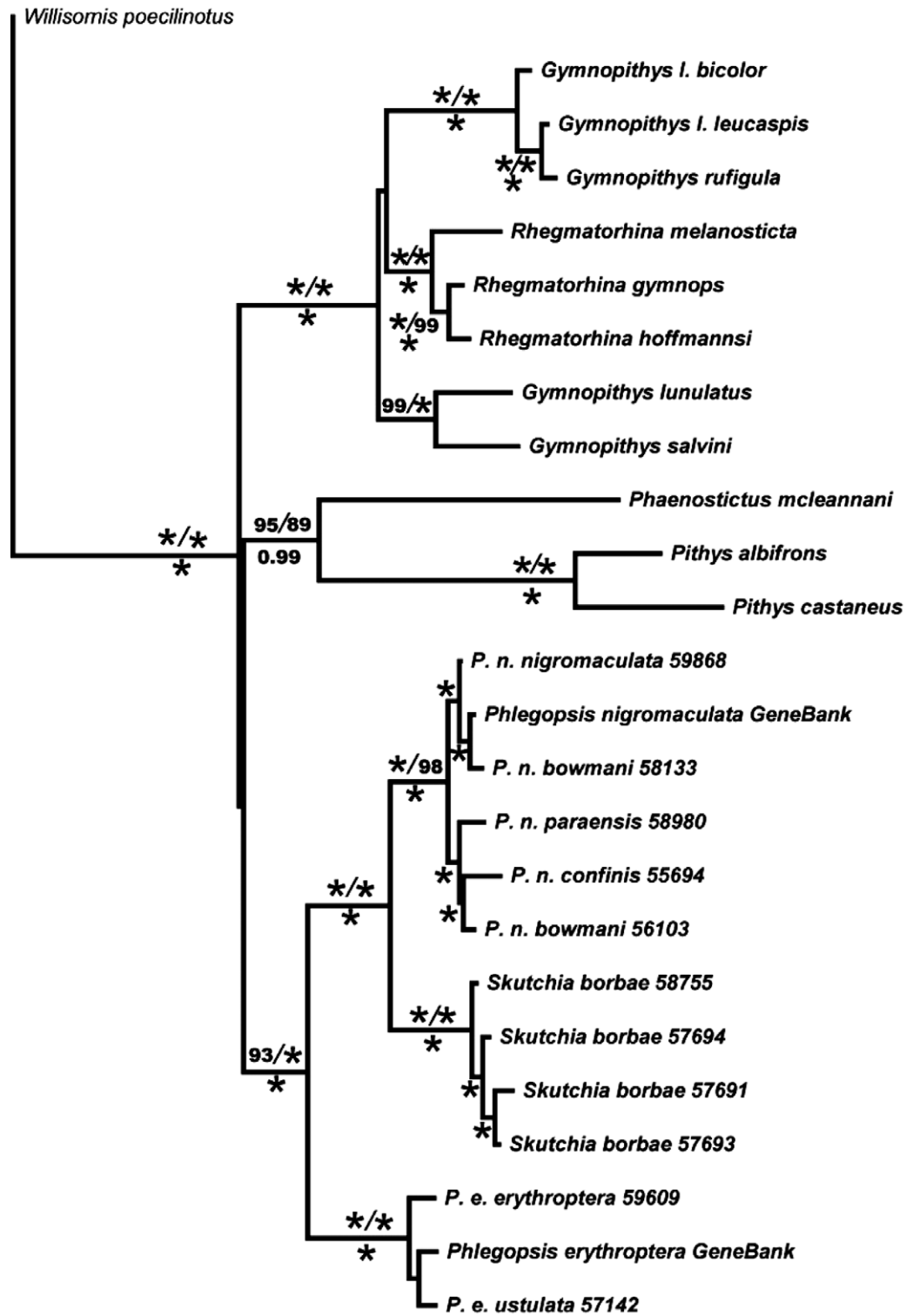


FIGURE 1. Maximum-likelihood estimate of the phylogeny produced by a heuristic search of the combined *cyt b*-ND2 data. Bootstrap support values obtained by MP (before slash) and ML (after slash), as well as Bayesian posterior probabilities (under the slash), are shown. Asterisks (*) denote nodes with bootstrap values for MP and ML of 100 and Bayesian posterior probabilities of 1.0. For clarity, only Bayesian posterior probabilities are shown for nodes pertaining to the relationships among *P. erythroptera*, *P. nigromaculata*, and *Skutchia*. Sequences of *Phlegopsis* downloaded from GenBank are labeled as such. Nodes with no numbers or asterisks were poorly supported, as indicated by bootstrap values $\leq 50\%$ and Bayesian posterior probabilities < 0.95 .

TABLE 3. Measures of homoplasy for plumage characters used by Willis (1968) to diagnose the genus *Skutchia*. Consistency index (CI) and retention index (RI) are for individual characters mapped onto the phylogeny of core obligate army-ant-following antbirds presented by Brumfield et al. (2007), where *Skutchia borbae* is placed as the sister taxon of *Phlegopsis nigromaculata* as shown in Fig. 1. Signal calculations were done by the method of Maddison and Slatkin (1991).

	Character	CI	RI	Signal (%) ^a
1	Black in the body plumage of males	0.25	0.25	29.6
2	Color intensity of the bare orbital skin	0.40	0.25	43.7
3	Patch of stiffened black feathers above the eye	0.50	0	100
4	Patch of elongated feathers on the lores	0.50	0	100
5	Barring on the chest and belly	0.33	0	100
	Overall index	0.37	0.33	

^aValues >5% indicate no significant phylogenetic signal.

and Isler 2003). A most unexpected result, however, was the clear sister relationship between *Phlegopsis nigromaculata* and *Skutchia* to the exclusion of *P. erythroptera*, rendering the genus *Phlegopsis* paraphyletic (Fig. 1). This finding is also supported by comparative levels of pairwise uncorrected sequence divergence, which average lower between *Skutchia* and *P. nigromaculata* (5.45%; range 4.78–6.06%) than between *P. nigromaculata* and *P. erythroptera* (7.69%; range 7.2–8.48%).

When Hellmayr (1907) described and placed *S. borbae* in the genus *Phlegopsis*, he was motivated primarily by an overall resemblance between the brown plumages of the holotype of *S. borbae* (a male) and of female *P. erythroptera*. In the original description, Hellmayr (1907) never mentioned *P. nigromaculata* (the actual sister species of *S. borbae*; Fig. 1) because in this species the male is predominantly black (Zimmer and Isler 2003). Willis (1968) speculated that Hellmayr (1907) tried to reconcile the brown male plumage of *S. borbae*, when he placed it in *Phlegopsis*, in which males of both *P. erythroptera* and *P. nigromaculata* are mostly black, by considering the holotype to be an immature, despite the lack of any evidence for this conclusion. In fact, Willis (1968) discussed evidence indicating that the holotype of *S. borbae* is an adult male. This apparent contradiction was the main factor prompting Willis (1968) to propose the genus *Skutchia*. Willis (1968) noted that he and other ornithologists had been misled by Hellmayr's (1907) original description to expect that the adult male of *P. borbae* would be black, and it was not until Willis rediscovered the species in 1966 that ornithologists realized the adult male is brown.

The phylogenies presented in this study (Fig. 1) and in Brumfield et al. (2007) provide a novel framework for the study of character evolution in the clade of obligate army-ant-following antbirds, allowing a critical re-evaluation of the taxonomic usefulness of the characters used to distinguish *Skutchia* from other genera and species in this group. When mapped onto molecular phylogeny, all plumage characters originally used to distinguish *Skutchia* from other lineages in the clade (especially *Phlegopsis*) are revealed as also shared by the closely related genera *Rhegmatorhina*, *Gymnophithys*, *Pithys*, and *Phaenostictus*, likely as a result of convergence and/or retention of ancestral characters, weakening the validity of *Skutchia* as a monotypic genus. On the other hand, sexual dimorphism in plumage color (not mentioned by Willis 1968) exhibits a strong phylogenetic signal when mapped onto phylogeny (CI = 0.33, RI = 0.67, $P = 0.03$; cladogram and character-state matrix available upon request), and represents a

shared character loss uniting *P. nigromaculata* and *S. borbae* to the exclusion of the strongly dimorphic *P. erythroptera*.

Inasmuch as generic limits are often drawn arbitrarily (see Willis 1968 for an interesting discussion on the subject pertaining to the obligate army-ant-following species) and are often difficult to define objectively, the data we present support the return of *S. borbae* to the genus *Phlegopsis*, where it was originally placed by Hellmayr (1907). Since the type species of *Phlegopsis* is *P. nigromaculata* (Gray 1855), merging *Skutchia* and *Phlegopsis* is a more conservative taxonomic approach to retaining *Skutchia* and erecting a new genus for *P. erythroptera*. This alternative is also at odds with levels of comparative genetic variation found in other genera of the core obligate army-ant-following clade, such as *Gymnophithys*, in which the range of uncorrected sequence-divergence levels (0.71–8.42%) is similar to that of the “inclusive” genus *Phlegopsis* as defined here (0.57–8.48%).

In conclusion, the genus *Skutchia* Willis, 1968, should henceforth be considered a junior synonym of *Phlegopsis* Reichenbach, 1850, which now includes three biological species in the following systematic sequence: *P. erythroptera* (Gould, 1855), *P. nigromaculata* (d'Orbigny and Lafresnaye, 1837), and *P. borbae* Hellmayr, 1907.

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