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A new species of African Forest Robin from Gabon (Passeriformes: Muscicapidae: *Stiphrornis*)

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Abstract

We describe a new species of forest robin from the Gamba Complex in southwest Gabon. This common bird, *Stiphrornis pyrrholaemus* **sp. nov.**, inhabits primary lowland forest and forages on or near the ground like the other members of the genus *Stiphrornis* of central and western Africa. Unique phenotypic features of the new species include the male's bright orange chin, throat, and breast, creamy yellow belly, olive green back and rump, and gray flanks. Mitochondrial sequence divergence corroborates our assessment based on its distinct physical characteristics that this is a new species, and suggest that *Stiphrornis erythrothorax* is likely the most closely related congener.

Key words: African forest robin, *Stiphrornis pyrrholaemus*, Gamba Complex, Moukalaba–Doudou National Park

Introduction

The tropical rainforests of central Africa remain one of the least scientifically explored regions of the world due to their remoteness, inhospitable environment, and density of forest cover. Yet, intensive logging and industrialization have begun to reduce and fragment this immense region of dense humid forest, savannah, and wetlands (Laporte, et al. 2007). Documenting the flora and fauna of this region, particularly the biologically diverse Gamba Complex of Protected Areas in Gabon, is a primary conservation priority.

In 2000, the Smithsonian Institution's Monitoring and Assessment of Biodiversity Program (SIMAB) and Shell International started to develop relationships among industry, governments and researchers that foster a more environmentally friendly approach to resource development and extraction, while promoting the conservation of biodiversity. With support from Shell Foundation and Shell Gabon, SIMAB enlisted biologists to help with the biodiversity assessment in the Gamba Complex of Gabon (Fig. 1). As part of this team, ornithologists from the Smithsonian Tropical Research Institute and National Museum of Natural History (USNM) conducted five surveys from 2001 to 2003 in the Gamba Complex (Angehr, et al. 2006).

During these surveys, specimens of forest robin (*Stiphrornis*) were obtained from two sites: Rabi Oil Field and our camp in the southwestern part of Moukalaba–Doudou National Park (Fig. 1). Upon comparing the materials with existing museum specimens, it became apparent the birds from these two sites were phenotypically different from the known species of *Stiphrornis*. Beresford and Cracraft (1999) suggested that the genus *Stiphrornis* is composed of four distinct species: *S. erythrothorax* Hartlaub, *S. gabonensis* Sharpe, *S. sanghen-*

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sis Beresford & Cracraft, and *S. xanthogaster* Sharpe. Two of these, *S. gabonensis* and *S. xanthogaster*, are known to occur in Gabon (Beresford & Cracraft 1999; Berlioz 1954; Malbrant & Maclatchy 1949). The birds obtained in the Gamba Complex did not match the physical descriptions of either of these known species. To confirm the phenotypic differences between these birds and other *Stiphrornis* species, we sequenced mitochondrial (cytochrome-*b*, ND5, and tRNA-thr) and nuclear (Glyceraldehyde-3-phosphate dehydrogenase and Beta-fibrinogen) regions and compared them to a phylogeny of other taxa of the clade developed by Beresford and Cracraft (1999). DNA sequences corroborated our assessment of the physical uniqueness of the Gamba birds. Hence, we propose to name this new species:

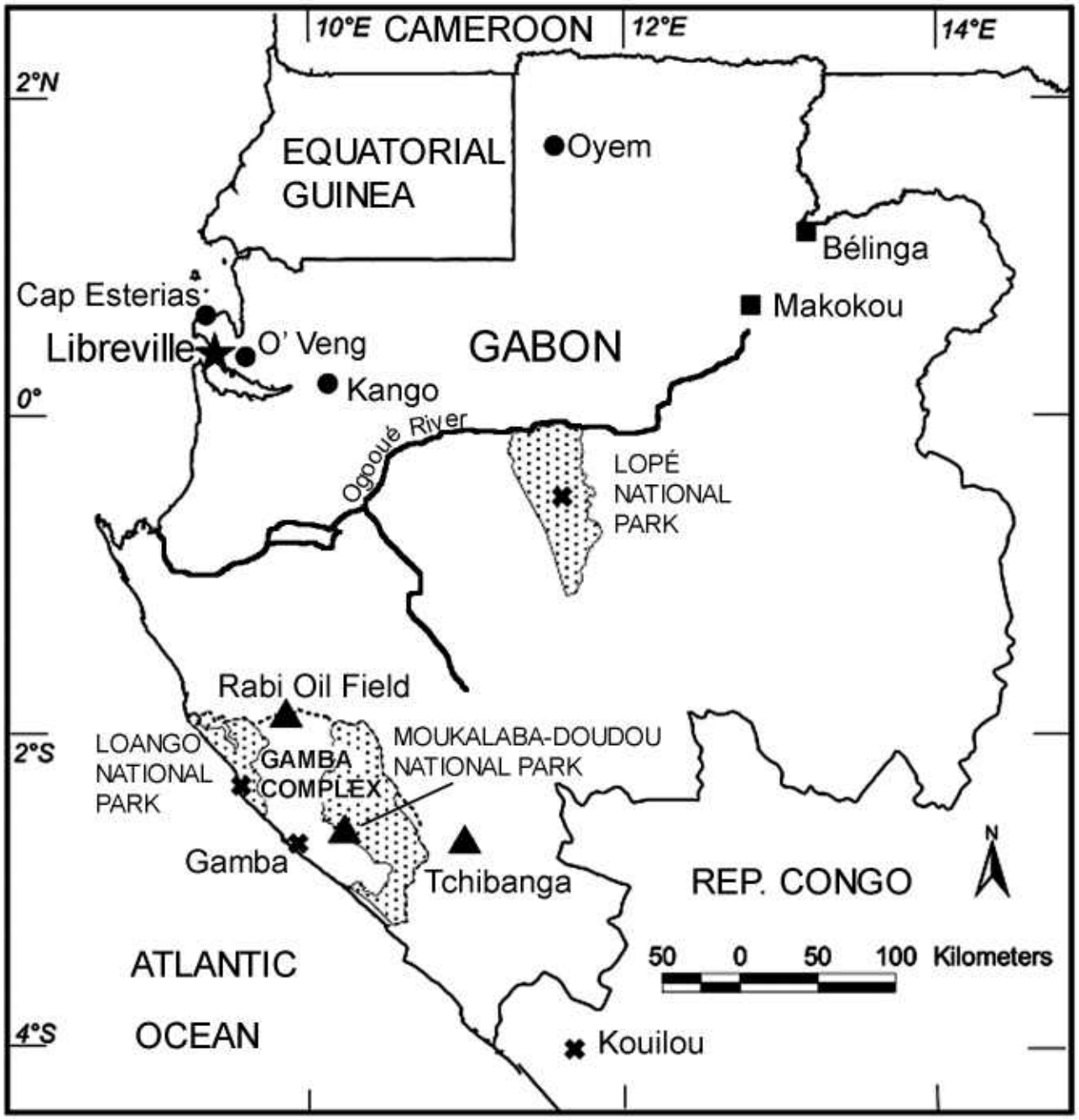

FIGURE 1. *Stiphrornis* records in Gabon and southern Republic of the Congo. Filled circles = *S. gabonensis,* filled squares = *S. xanthogaster,* filled triangles = *S. pyrrholaemus,* crosses = *Stiphrornis* recorded but species identity unknown or uncertain.

***Stiphrornis pyrrholaemus*, sp. nov. (Schmidt & Angehr)**
Olive-backed Forest Robin (English)
Rougegorge de forêt à dos olive (French)

Holotype—USNM 631622; adult male (skull 100% pneumatized and no bursa), from northwest corner of N'dogo Lagoon within Moukalaba–Doudou National Park, Ogooue Maritime Province, Gabon (2°25'14"S, 10°14'04"E); elevation < 200m; collected on 14 Apr 2003 by Brian K. Schmidt (BKS 6309, tissue B15037) and prepared by him as a study skin, tissue preserved in Seutin buffer solution (Seutin, et al. 1991), and trunk preserved in 3% formalin. Mitochondrial and nuclear DNA sequences were deposited in GenBank (accession no. EU423168).

Diagnosis—Typical of other members of the genus *Stiphrornis* with its fine bill and weak rictal bristles, white loral spot, long legs, short tail, and rich orange on the throat and chest (Irwin & Clancey, 1974). Distinguished from *S. gabonensis, S. xanthogaster*, and *S. erythrothorax* by its yellow belly, and differs from *S. sanghensis* (only other member of the genus with prominent yellow belly) by its olive-green back, rump, and upper tail coverts, and its darker orange throat (Table 1).

TABLE 1. Summary of plumage variation in *Stiphrornis* (modified from Beresford & Cracraft 1999).

Plumage/body part	*pyrrholaemus*	*sanghensis*	*xanthogaster*	*gabonensis*	*erythrothorax*
Forehead, forecrown, and crown	Dark gray to black	Gray to dark gray with olive wash	Gray to dark gray with olive wash	Darker gray with faint olive wash	Gray with green wash
Nape, mantle, and back	Olive green	Gray with olive wash	Gray with olive wash	Slaty gray, only faintly tinged olive	Gray with green wash
Chin, throat, and breast	Bright orange	Bright yellow orange	Tawny; varies to pale beige at chin and throat	Russet	Russet
Upper and lower belly	Cream Yellow	Yellow	Cream	White	White
Upper wing coverts	Gray with olive fringes	Dark brown	Dark brown	Dark brown to slaty gray	Dark brown edges olive
Rump, upper tail coverts and dorsal surface of rectrices	Olive Green	Gray washed yellow-green	Gray with olive wash generally brighter than on back	Gray with olive wash generally brighter than on back	Gray with green wash, not distinct from back
Lesser underwing coverts	Gray tipped white	Gray tipped yellow	Gray tipped cream	Gray tipped white	Gray tipped white
Flank and tibiotarsus	Gray	Light gray tipped yellow	Light gray tipped cream	Light gray tipped white	Light gray tipped white

Description of holotype—Capitalized color designations (corresponding number in parentheses) from Smithe (1975). Forehead and crown Blackish Neutral Gray (color 82). Nape dark gray with slight Olive-Green (aux. 48) wash increasing dorsally. Back and rump Olive-Green (aux. 48). Rectrices Fuscous (color 21) fringed slightly browner than rump and same color as the upper tail coverts. Remiges Brownish-Olive (color 29) narrowly fringed with Olive-Green (aux. 48). Wing coverts gray with olive fringing. Wing lining whitish and axillaries creamy yellow. Malar and eye ring black. Loral spot white. Auricular black blending to Blackish Neutral Gray (color 82) posteriorly. Chin and throat Spectrum Orange (color 17). Upper breast dark orangish ochre with feather fringes on sides of breast Medium Neutral Gray (color 84). Lower breast and belly creamy yellow with under tail coverts slightly darker. Flanks and sides Medium Neutral Gray (color 84). Soft parts in life: iris dark brown, bill black, legs and feet pink-gray (Figs. 2, 3).

Measurements of holotype—Wing chord 68.7 mm; tail 41.1 mm; culmen from base of feathers 14.1 mm; bill width at anterior edge of nares 3.6 mm; bill depth at anterior edge of nares 4.05 mm; tarsus 24.7 mm; mass 20.5 g; skull 100% pneumatized; left testis 2 x 1.5 mm, gray in color.

Description of allotype—USNM 631537; adult female (skull 100% pneumatized, no bursa) from northwest corner of N'dogo Lagoon within Moukalaba–Doudou National Park, Ogooue Maritime Province, Gabon (2°25'14"S, 10°14'04"E); elevation < 200m; collected on 31 Mar 2003 by Brian K. Schmidt (BKS 6224, tissue B16312) and prepared by him as a study skin, tissue preserved in Seutin buffer solution, and trunk preserved in 3% formalin. Mitochondrial and nuclear DNA sequences were deposited in GenBank (accession no. EU423171). Forehead and crown gray with slight Olive-Green (aux. 48) wash. Back and rump Olive-Green (aux. 48). Rectrices Fuscous (color 21) fringed slightly browner than rump and same color as the upper tail coverts. Remiges slightly lighter than Brownish-Olive (color 29) narrowly fringed with Olive-Green (aux. 48) in the holotype. Wing coverts gray with olive fringing. Wing lining whitish and axillaries creamy yellow. Malar and eye ring Medium Neutral gray (color 84). Loral spot white. Auricular black blending to Blackish Neutral Gray (color 82) posteriorly. Chin and throat Orange Yellow (color 18). Upper breast light orangish ochre with feather fringes on sides of breast Medium Neutral Gray (color 84). Lower breast and belly cream colored (not as yellow as holotype) with under tail coverts slightly darker. Flanks and sides Medium Neutral Gray (color 84). Soft parts in life: iris dark brown, bill black, legs and feet pink-gray (Figs. 2, 3).

Measurements of allotype—Wing chord 60.4 mm; tail 35.3 mm; culmen from base of feathers 14.1 mm; bill width at anterior edge of nares 3.8 mm; bill depth at anterior edge of nares 4.2 mm; tarsus 23.1 mm; mass 17.5 g; skull 100% pneumatized; ovary 6 x 3 mm, granular.

TABLE 2. Morphological measurements of type series. Measurements are in millimeters, weight in grams, and missing measurements are skeleton preps.

USNM	Sex	Weight	Wing	Tail	Culmen	Bill Depth	Bill Width	Tarsus
631537	F	17.5	60.4	35.3	14.1	4.2	3.8	23.1
616804	M	17.0	61.9	28.2	14.4	3.7	3.8	24.6
631495	M	19.0	-	-	-	-	-	-
631496	F	19.0	-	-	-	-	-	-
631501	M	18.0	67.6	39.5	13.0	3.7	3.7	25.1
631584	F	17.0	-	-	-	-	-	-
631486	M	19.0	-	-	-	-	-	-
631511	M	18.5	62.3	41.5	13.3	3.6	3.6	24.8
631520	F	16.0	-	-	-	-	-	-
631524	F	18.0	64.2	32.4	12.6	3.9	3.6	24.1
631622*	M	20.5	68.7	41.1	14.4	4.05	3.6	24.7
616712	F	18.5	61.9	28.2	13.4	3.7	3.6	24.5

* type specimen.

Paratypes—There are a total of ten additional specimens from the type series. One skin specimen has been returned to Gabon for their collections (USNM 631501) and the remaining nine are deposited at USNM with associated tissue samples (B numbers) preserved in Seutin buffer solution. Two specimens from Rabi Oil Field: female, fluid preserved, USNM 616712, B10005; and male, fluid preserved, USNM 616804. Eight specimens from Moukalaba–Doudou National Park: male, skeleton, USNM 631486, B16281 (Fig. 3); male, skeleton USNM 631495, B16285; female, skeleton, USNM 631496, B16286; male, skin and fluid preserved trunk, USNM 631501, B16291 (housed at the Smithsonian's biodiversity lab at Vembo near Gamba, Gabon, until adequate facilities are available for permanent storage in Gabon); male, fluid preserved, USNM 631511;

female, skeleton, USNM 631520, B16300; female, skin and fluid preserved trunk, USNM 631524, B16304; and female, skeleton, USNM 631584, B16347.

Measurements and color of type series—Morphological measurements of the type series (Table 2) were taken with digital calipers and rounded to the nearest 0.1 mm: wing chord; tail (from point of insertion to tip of central retrix); culmen (from anterior extension of feathers); bill depth at anterior edge of nares; bill width at anterior edge of nares; and tarsus. Weights to the nearest 0.1 gram were obtained immediately after capture by using a Pesola spring scale to weigh the bird in the cloth bag and then subtracting bag weight.

Color measurements from the type series were obtained using a calibrated colorimeter (CR-221 Chroma Meter, Minolta Corporation) following procedures described by Graves (1999). The procedure was repeated five times for each area of plumage. Each datum summarized in Table 3 represents five independent measurements. Colorimetric characters were described in terms of opponent-color coordinates (*L*, *a*, *b*) (Hunter 1987). This system uses three coordinates to express colors. The *L* coordinate, ranging from 0 to 100, describes the "lightness" of color; low values are dark and high values are light. The *a* coordinate describes the "redness" and "greenness"; reds have positive value and greens negative value. The third coordinate *b* describes the "yellowness" and "blueness"; yellows have positive value and blues negative value. These measurements confirm visual differences between the males and females: males have darker orange throat, darker head, and yellower belly. Both sexes share similar back coloration.

Other specimens—Only one other *S. pyrrholaemus* specimen has been identified outside the type series. A juvenile unsexed skin at the Muséum d'Histoire Naturelle de Paris (NMHN 1954-56) was collected by P. Rougeot on 11 Nov 1953 in Tchibanga, Gabon. Identification was confirmed using ancient DNA techniques (see phylogenetic relationships section below). Berlioz (1954) noted that the upperparts were a deep olive brown (*brun olivâtre foncé*) and the entire underparts strongly tinted yellow (*fortement teinté de jaune*), with an intense but poorly defined area of russet (*roussâtre*) on the breast. The yellow on the specimen has obviously faded since Berlioz's description as the underparts are currently white with a hint of yellow and the breast a dull russet. The throat is slightly more yellow than the underparts and the feathers are sparsely tipped gray which is usually a juvenile trait. Likewise, the olive gray wing coverts and tertials have light brown tips giving a spotted appearance which is also a juvenile trait in this genus. The forehead, crown, and nape are lighter gray than in adults and lacking the olive wash. The white loral spot is not as prominent as in adult birds. Berlioz doubtfully assigned it to *S. xanthogaster* because of its strongly yellowish underparts, but believed that this coloration could have been a juvenile trait as in some other thrushes. He also remarked that if this specimen were actually *xanthogaster,* it would be "paradoxical" to find the subspecies with yellow underparts known from southeast Cameroon, instead of that with a white abdomen known from western Cameroon and Gabon. Berlioz's (1954) bewilderment is understandable now that we know this specimen is actually a member of the new species.

Etymology—The brilliant orange 'flame' colored throat of this species outshines the others in the genus. The *pyrrho-* prefix signifies 'orange colored' and the *–laemus* suffix refers to 'the throat'. The combined Greek name is meant to describe: A stout/sturdy bird (*Stiphrornis*) that bears a flame colored throat (*pyrrholaemus*). Combined with the English common name of Olive-backed Forest Robin which highlights the distinctive olive back and rump, the bird is aptly described by its names.

Distribution—The full distributional range of the new species is not known. In addition to our specimens and observations from the Rabi Oil Field (1°52'S, 9°53'E), along the road to Toucan (1°51'S, 9°51'E) immediately north of Rabi, and Moukalaba–Doudou National Park, and the 1953 specimen from Tchibanga (2° 51'S, 11° 01'E), we found *Stiphrornis* at two other localities in the Gamba Complex: near Gamba (2°45'S, 10°01'E) and near our base camp in Loango National Park (2°20'S, 9°35'E) (Fig. 1; see Angehr et al. 2005, 2006 for descriptions of these localities). We obtained tape recordings at the Gamba and Loango sites but did not capture, photograph, or observe individuals closely enough there to be certain of their species identity. Based on calls, we found *Stiphrornis* to be common at Gamba and our camp at Moukalaba–Doudou, and uncommon in the Rabi–Toucan area and our camp in Loango National Park (Angehr, et al. 2005).

TABLE 3. Ranges and mean (± standard deviation) of opponent color coordinates (L, a, b) reflected from plumage areas of type series specimens. L (Lightness), *a* (Red[+]/Green[-]), *b* (Yellow [+]/Blue[-])

	USNM 631622* ♂	**USNM 631501** ♂	**USNM 631524** ♀	**USNM 631537** ♀
Upper Throat				
L	56.6-62.1 59.9±2.2	60.1-64.3 62.8±2.1	63.9-66.0 65.1±0.8	69.5-72.8 71.8±1.3
a	17.3-23.7_21.8±2.6	18.5-23..7 20.7±2.3	16.2-18.6 17.3±0.9	4.8-7.1 5.7±0.9
b	66.9-74.7 70.0±3.3	69.7-73.1 71.1±1.4	68.6-72.8 71.2±1.7	65.2-68.0 66.7±1.2
Crown				
L	22.6-23.1 22.8±0.2	20.3-22.0 21.2±0.8	23.6-24.2 23.8±0.3	23.2-25.0 24.1±0.7
a	0.1-0.5 0.3±0.1	0.2-0.6 0.4±0.2	-0.1-(+0.2) 0.1±0.1	0.2-0.4 0.3±0.1
b	1.3-2.7 2.2±0.7	1.9-2.7 2.3±0.3	7.2-7.6 7.4±0.2	3.9-4.9 4.6±0.5
Back				
L	27.0-28.0 27.5±0.5	27.6-29.1 28.4±0.7	27.0-28.1 27.4±0.4	27.6-28.6 28.0±0.4
a	-0.9-(+1.3) 0.1±0.8	-0.8-(-0.5) -0.6±0.2	-0.4-(+0.2) -0.6±0.2	0.2-0.6 0.5±0.2
b	9.1-14.0 11.8±2.0	13.8-16.4 15.1±1.2	12.1-13.3 12.6±0.5	12.6-15.9 14.2±1.5
Belly				
L	80.2-82.2 81.1±0.8	80.7-83.3 82.0±1.2	81.8-84.3 82.5±1.8	79.4-81.4 80.6±0.8
a	-3.1-(-0.8) -2.2±0.9	-2.7-(-1.7) -2.4±0.4	-3.2-(-1.9) -2.7±0.6	-3.6-(-3.3) -3.5±0.1
b	40.3-45.3 42.9±1.9	32.8-35.9 34.3±1.3	21.2-31.6 27.8±4.3	22.3-28.0 25.4±2.7

* type specimen.

There are few published specimen records of *Stiphrornis* for Gabon (Fig. 1). As noted by Beresford and Cracraft (1999), records of *S. gabonensis* are limited to north of the Ogooué River, including Kango (Malbrant & Maclatchy 1949), Cap Esterias (Rand, et al. 1959) and O'Veng (Berlioz 1955), all in the vicinity of Libreville, and near Oyem in Woleu–N'Tem province (Rougeot 1951), the last apparently the farthest inland. Records of *S. xanthogaster* from Gabon are limited to Bélinga and Makokou in the northeast (Beresford & Cracraft 1999; Brosset & Erard 1986, specimens in Muséum d'Histoire Naturelle de Paris). The only record of *Stiphrornis* south of Gamba and Tchibanga is from the Kouilou area of the Mayombe region of Republic of the Congo (Dowsett-Lemaire & Dowsett 1991). Although Dowsett-Lemaire and Dowsett found *Stiphrornis* to be common, they did not note subspecies (as the known taxa were then treated) or describe the plumage.

Specimens from central Gabon will be of great interest in elucidating the relationships between the different forms. It is of interest to note that Christy and Clarke's (1991) description and illustrations of birds from

the Lopé National Park suggest either that two different forms are present there, or that the population is intermediate and variable. They describe the species as having dark slate-gray upperparts, orange-yellow to bright orange underparts and a pure white belly, with some individuals having a brilliant orange throat and a citron-yellow breast. Their illustration shows birds with entirely gray upperparts, yellow-orange throat and upper breast, bright yellow lower breast, and white belly. Likewise, a photograph of a *Stiphrornis* by Don Roberson in 1996 (pers. comm.) from Lopé with orange throat and yellow belly might also suggest that the new species occurs north to Lopé, but the bird in the photo cannot be identified conclusively.

Remarks

Behavior and ecology—Stiphrornis pyrrholaemus is a locally common bird, but like other members of the genus it is unobtrusive and its presence is made known mainly by its call. Our visual observations were mainly in response to playback of vocalizations. Its ground dwelling behavior is typical of other members of this genus. Observations and encounters were mostly in primary lowland forest with light to moderate undergrowth. In open primary forest with heavy grazing by elephant and forest buffalo that greatly reduces undergrowth, the bird appears uncommon or even absent. Near Gamba, we found it to be most common in the area of the Totou well sites (2°42'03"S, 10°00'01"E), which had continuous and less fragmented forest than other sites we studied, and apparently rare or absent in areas of more secondary or disturbed forest. Sargeant (1993) reported *Stiphrornis* to be present at five additional sites in the Gamba area, it being most common in the well-forested region near Pont Brulé, ca. 20 km east of Gamba (2°42'S, 10°18'E). Near Rabi Oil Field, where the species was generally uncommon, we most frequently encountered it along the road to Toucan, which penetrated less disturbed forest. The species was least frequently encountered near our Loango camp, where elephants and large ungulates were common and undergrowth sparse. It was most common (encountered daily on various transects, and/or netted every day) near the Mont Doudou camp, where elephants and large ungulates were scarce and undergrowth was relatively abundant.

Vocalizations—We obtained two recordings of adequate quality and length to produce spectrograms (Fig. 4). Beresford and Cracraft (Beresford & Cracraft 1999) identified two types of vocalizations in *Stiphrornis*. Their "Type A" begins with a few high chirps and continues with a series of modulated notes. "Type B" is a rolling trill (lacking clear demarcation of units or phrases in *sanghensis*) without introductory notes. Our recordings are similar in structure to these vocalization types, but differ in detail. The Type A vocalization recorded for *pyrrholaemus* begins with a single chirp, rather than the several described for *sanghensis* and *xanthogaster* (Beresford & Cracraft 1999), and some phrases terminate with a single chirp after the modulated portion (first and third sequences in the spectrogram), the latter feature not described for other *Stiphrornis*. The Type B vocalization consists of continuously repeated phrases, unlike that of *sanghensis*. Examples of Type B vocalizations of *xanthogaster* shown in Beresford and Cracraft (1999) have continuously repeated phrases, but these differ in structure from our example of *pyrrholaemus*.

Beresford and Cracraft (1999) did not include vocalizations of *erythrothorax* or *gabonensis* in their analysis. Keith et al. (1992) onomatopoetically describe vocalizations of *Stiphrornis* from Sierra Leone, Gabon, Zaire, and Uganda as each being different, and Borrow and Demey (2001) describe the vocalizations of *gabonensis* and *xanthogaster* as differing. Chappuis (2000) includes recordings of vocalizations from Ivory Coast (*erythrothorax*) and Makokou, Gabon (*xanthogaster*). The Ivory Coast vocalization consists of well spaced, variable phrases without introductory notes. The Makokou recording is of Type A vocalizations, each with two to three introductory notes.

FIGURE 2. *Stiphrornis pyrrholaemus* watercolor by John Anderton. Male above, female below.

FIGURE 3. Above: handheld photographs of male *Stiphrornis pyrrholaemus* from Moukalaba–Doudou National Park (USNM 631486). Bottom: Holotype specimen USNM 631622 (top) and allotype USNM 631537 (bottom). Photos by Brian K. Schmidt.

As noted by Beresford and Cracraft (1999) the extent to which these differences in vocalizations represent variation among individuals, populations, or species is at present unknown. More extensive recordings from populations whose species identity has been confirmed will be necessary to determine the full vocal repertoire of each *Stiphrornis* species.

Phylogenetic relationships

Laboratory techniques—DNA was extracted from pectoral muscle using Qiagen DNeasy® kits following standard tissue extraction protocols. Fragments of mitochondrial (mt) and nuclear DNA were amplified using

polymerase chain reaction (PCR) and we purified products with QIAquick PCR kits (Qiagen, Valencia, CA). 1 µl of PCR product was cycle sequenced in a 10 µl reaction, the resulting product was gel filtered using Sephadex (Sigma–Aldrich, St. Louis, MO), and run in an ABI 3100 automated sequencer. For mtDNA, four segments were amplified using the primer pairs of Beresford and Cracraft (1999), 1143 bp of the cytochrome-*b* gene, 315 bp of the adjacent ND5 gene, and 28 bp of the tRNA-thr gene, for a total of ~1486 bp. PCR reactions also followed protocols of Beresford and Cracraft (1999), except we used 20 µl reactions and the following thermocycle profile: 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, extension at 72°C for 1 min, and a final extension of 72°C for 7 min. Nuclear DNA of the introns of two genes, Glyceraldehyde-3-phosphate dehydrogenase (Gapd) and Beta-fibrinogen (intron 5, fib 5) were amplified. For Gapd, a 605 bp fragment was targeted with the primers GapdL890 and GapdH950 following the protocols of Friesen et al. (1997). For fib 5, a 550 bp fragment was amplified with primers Fib5 and Fib6 and followed protocols of Fuchs et al. (2004).

In addition, DNA was isolated from a toe pad sliver sampled from the specimen of unidentified, immature *Stiphrornis* from the Paris Museum (MNHN 1954-56) using standard phenol-chloroform and centrifugal dialysis methods (Fleischer et al. 2001). At the same time, a no-tissue tube was extracted as a negative control. All extraction and PCR setup was conducted in an isolated ancient DNA laboratory to minimize opportunity for contamination from modern DNA or PCR products. Amplifications of two smaller pieces of the cytochrome b gene were attempted from these extracts using primers Cytb1 and Cytb2, and Cytb2rc and Cytb-wow (see Fleischer et al. 2006 for primer sequences) and the PCR conditions cited above except that 55 cycles were used. Products were sequenced as outlined above.

Sequences for mtDNA analyses were generated from *Stiphrornis* spp. from specimens at the National Museum of Natural History (USNM) and the Academy of Natural Sciences of Philadelphia (ANSP), or obtained from GenBank from the work of Beresford and Cracraft (1999). In addition, Gapd and fib 5 nuclear regions were amplified from tissue from these same birds and included tissue from the individuals analyzed by Beresford and Cracraft (1999) obtained from the American Museum of Natural History. Novel sequences have been submitted to GenBank (Table 4).

Phylogenetic analyses—Sequences were edited and aligned in SEQUENCHER (Gene Codes Corporation, Ann Arbor, MI). The 315 base pair ND5 and the 28 base pair tRNA-thr segments were not present in three of the species (9 birds) obtained from GenBank, including a single *S. erythrothorax* and all samples from *S. gabonensis*, *S. xanthogaster*, and *Muscicapa infuscata*. Trees built with a reduced dataset (i.e., 1143 bp of cyt b only) did not differ from the ones shown here. Phylogenies were constructed using the mtDNA sequences based on maximum parsimony (MP), maximum-likelihood (ML), and Bayesian approaches. For the latter two approaches, MODELTEST (Posada & Crandall 1998) was used to estimate parameters of nucleotide substitution and to select the best model based on Akaike's Information Criterion (AIC). The best-fit model was General Time Reversible with a gamma distribution of 0.0797. Uncorrected *p*-distances and standard errors (bootstrap of 500 replications) were generated in MEGA (Kumar, et al. 2004). MP and ML analyses were run in PAUP* (Swofford 2002) and Bayesian analyses were conducted in MRBAYES (Huelsenbeck & Ronquist 2001). For MP analysis, heuristic tree searches were conducted with 10 random addition repetitions and a bootstrap analysis of 1000 replications. For ML analysis, we ran heuristic searches with 100 bootstrap replications. For Bayesian analysis, 1 million generations of Markov chain Monte Carlo simulations were used to approximate the posterior probabilities of trees with sampling frequency of every 100 trees. We had a 25% burn-in (i.e. the first 2500 trees were discarded) and maintained the default parameters of 4 chains, a temperature of 0.5 and uniform priors set to 1.0. Stationarity was reached during the burn-in based on the graph of the –ln likelihood values of the sampled generations. We generated a consensus topology from the remaining 7500 sampled generations. All comparisons were made to the outgroup taxon *Muscicapa infuscata*. Previous work had used *Sheppardia cyornithopsis* (Beresford & Cracraft 1999) but subsequent cyt b analyses indicated that *Muscicapa infuscata* is likely more closely related to *Stiphrornis* (Beresford 2003).

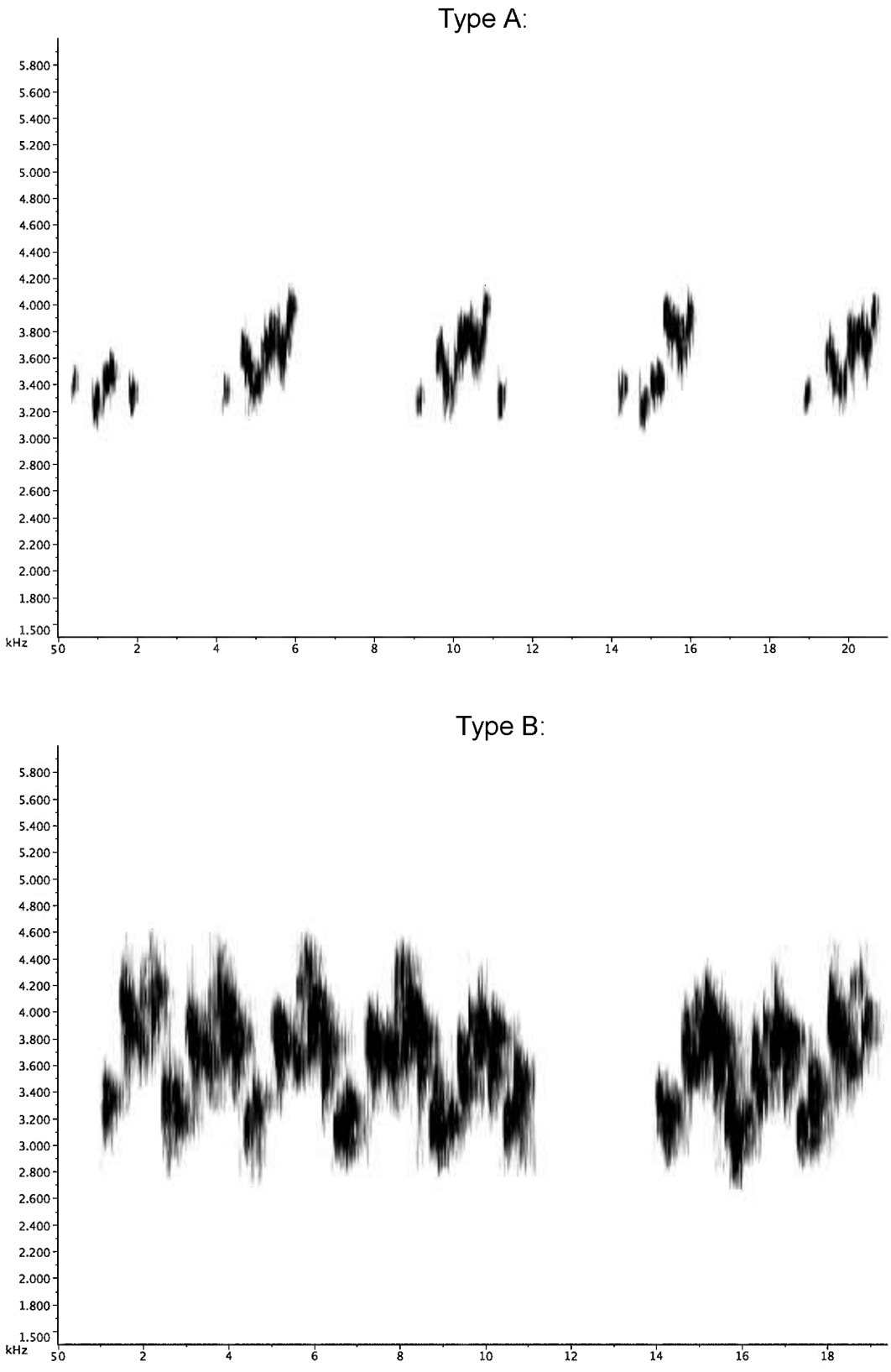

FIGURE 4. Spectrograms of vocalizations of *S. pyrrholaemus.* See text for description of vocalization types. Type A recorded 11 March 2002, Type B 11 June 2002, both on Toucan Road immediately north of the Rabi Oil Field, by George R. Angehr.

TABLE 4. *Stiphrornis spp.* samples used in this study. Tissue from unknown *Stiphrornis* sp. came from birds collected in Gabon, *S. gabonensis* specimens from ANSP were collected in Equatorial Guinea. Cytochrome b (cyt B) region includes portions of the genes ND5 and tRNA-thr. Nuclear genes β fibrinogen and Gapd not shown (EU423136–423157) because no amplification occurred in *S. pyrrholaemus* samples.

Species	Specimen number[a]	Collection locality	GenBank accession number cyt B region
S. pyrrholaemus	USNM 616712	Gabon	EU423167
S. pyrrholaemus	USNM 631486	Gabon	EU423174
S. pyrrholaemus	USNM 631495	Gabon	EU423175
S. pyrrholaemus	USNM 631496	Gabon	EU423176
S. pyrrholaemus	USNM 631501	Gabon	EU423169
S. pyrrholaemus	USNM 631520	Gabon	EU423170
S. pyrrholaemus	USNM 631524	Gabon	EU423172
S. pyrrholaemus	USNM 631537	Gabon	EU423171
S. pyrrholaemus	USNM 631584	Gabon	EU423173
S. pyrrholaemus	USNM 631622	Gabon	EU423168
S. pyrrholaemus	MNHN 1954-56	Gabon	EU826535
S. gabonensis	ANSP 11576	Equatorial Guinea [b]	EU423179
S. gabonensis	ANSP 11578	Equatorial Guinea [b]	EU423180
S. gabonensis	ANSP 11733	Equatorial Guinea [b]	EU423177
S. gabonensis	ANSP 11734	Equatorial Guinea [b]	EU423178
S. gabonensis	ANSP 11918	Equatorial Guinea [c]	EU423182
S. gabonensis	ANSP 12204	Equatorial Guinea [c]	EU423181
S. gabonensis	ANSP 12256	Equatorial Guinea [c]	EU423183
S. erythrothorax	AMNH 827588	Liberia	AF136724[d], EU423160
S. erythrothorax	AMNH 827589	Liberia	AF136725[d], EU423161
S. sanghensis	AMNH 10836	Central African Republic	AF136731[d], EU423164
S. sanghensis	AMNH 24731	Central African Republic	AF136732[d], EU423165
S. sanghensis	AMNH 831845	Central African Republic	AF136729[d], EU423162
S. sanghensis	AMNH 831846	Central African Republic	AF136733[d], EU423166
S. sanghensis	AMNH 831847	Central African Republic	AF136730[d], EU423163

[a] Tissues came from the following museums: Academy of Natural Sciences, Philadelphia (ANSP), American Museum of Natural History (AMNH), National Museum of Natural History (USNM), Muséum d'Histoire Naturelle de Paris (MNHN).

[b] Collected at Centro Sur, Rio Lobo at Asoc.

[c] Collected at Wele-Nzas, Altos de Nsork.

[d] Sequenced by Beresford and Cracraft 1999, see for details.

Results

We sequenced 1486 base pairs of the mtDNA cytochrome *b* and portions of ND5 and tRNA-thr regions from 17 birds, including ten birds collected in Gabon and seven birds collected in Equatorial Guinea. All birds had been tentatively identified as *S. erythrothorax*. Sequences were generated for ten birds for fib 5, including five

S. sanghensis samples from AMNH, and were compared to *S. gabonensis* and *S. erythrothorax* sequences from GenBank. Four polymorphisms and a 10 bp deletion were common among all five samples of *S. sanghensis*, but the additional nine single base substitutions in the sequence for the other eight samples did not appear to be taxonomically informative. In the intron Gapd, a single substitution occurred in 14 sequences, and this difference separated *S. gabonensis* from the remaining *Stiphrornis* spp. Poor amplification of these introns prevented analysis of all samples; all *S. pyrrholaemus* samples failed to amplify for both introns. Limited variability within the existing intron sequences suggested that additional sequencing would not provide additional variation. Concatenation of intron sequences to mtDNA sequences and subsequent analysis did not improve tree resolution, apart from further differentiating *S. sanghensis* from other *Stiphrornis* spp. (data not shown).

PCR products were generated from the Paris *Stiphrornis* museum specimen extracts using the two Cytb primer sets noted above, and 510 bp of sequence was obtained. This sequence was aligned to the existing sequences we generated and those from GenBank, and was found to match exactly the sequence from *Stiphrornis pyrrholaemus* specimens USNM616712, 631524 and 631537 (Figure 5), and was within 1-2 bp of all other sequences derived from *Stiphrornis pyrrholaemus* specimens. On the other hand, it differed by 26-30 bp from sequences obtained from the other species of *Stiphrornis*. These results indicate that the previously uncertain Paris specimen is a juvenile *Stiphrornis pyrrholaemus*.

Tree constructions from MP, ML, and Bayesian analyses of mtDNA sequences all resulted in the same topology (Fig. 5). The recently collected *Stiphrornis* taxon from Gabon is clearly differentiated from its likely closest relative *S. erythrothorax*, with strong nodal support for each species in all analyses. Pairwise comparisons also indicate significant differentiation between this species and other *Stiphrornis* spp. (Table 5).

TABLE 5. Uncorrected pairwise differences (below diagonal) and standard errors (above diagonal) in cytochrome b region mtDNA between *Stiphrornis* species. Comparisons based on 30 samples grouped by species, with sample sizes following species names.

	1	2	3	4	5
1 *Stiphrornis pyrrholaemus* (10)	-	0.010	0.011	0.011	0.012
2 *Stiphrornis erythrothorax* (2)	0.054	-	0.011	0.011	0.012
3 *Stiphrornis gabonensis* (10)[a]	0.057	0.058	-	0.011	0.011
4 *Stiphrornis sanghensis* (5)	0.060	0.060	0.064	-	0.006
5 *Stiphrornis xanthogaster* (3)	0.067	0.064	0.063	0.028	-

[a]Sequences of birds from Equatorial Guinea (ANSP) combined with GenBank sequences.

Birds collected in Equatorial Guinea, where the species identification was uncertain, cluster with the species *S. gabonensis* (Fig. 5). MP and ML analyses suggest minor differentiation between these samples and known sequences of this species from GenBank. Uncorrected *p*-distances between known *S. gabonensis* samples and these samples indicate a mean distance of only 0.004 ± 0.001 SE, but differences of 0.051 ± 0.007 or greater for comparisons with other *Stiphrornis* species.

Discussion

MtDNA sequence data support the conclusion that the birds recently collected in Gabon represent a new species that is clearly differentiated from other forest robins in west and central Africa. This new species appears most closely related to *S. erythrothorax*, whose range extends across West Africa, from Sierra Leone to the Cameroon highlands of Nigeria and Cameroon (Beresford & Cracraft 1999). This relationship is surprising

due the close proximity of *S. pyrrholaemus* in Gabon to *S. gabonensis* in neighboring Equatorial Guinea and *S. sanghensis* in the Central African Republic. Cytochrome b sequence differences of at least 5.4% (± 1.0) between *S. pyrrholaemus* and other *Stiphrornis* are as great as the divergence between many closely related bird species (Klicka & Zink 1997; Lovette, et al. 1998). For a rough estimate of the timeframe of divergence, a mtDNA molecular clock calibration with a rate of change at 1.6 – 2.0% per million years (My) (Fleischer, et al. 1998; Klicka & Zink 1997; but see Lovette 2003) would indicate divergence of 2.7 – 3.4 My between *S. pyrrholaemus* and *S. erythrothorax* and 2.9 – 3.6 My between *S. pyrrholaemus* and *S. gabonensis*.

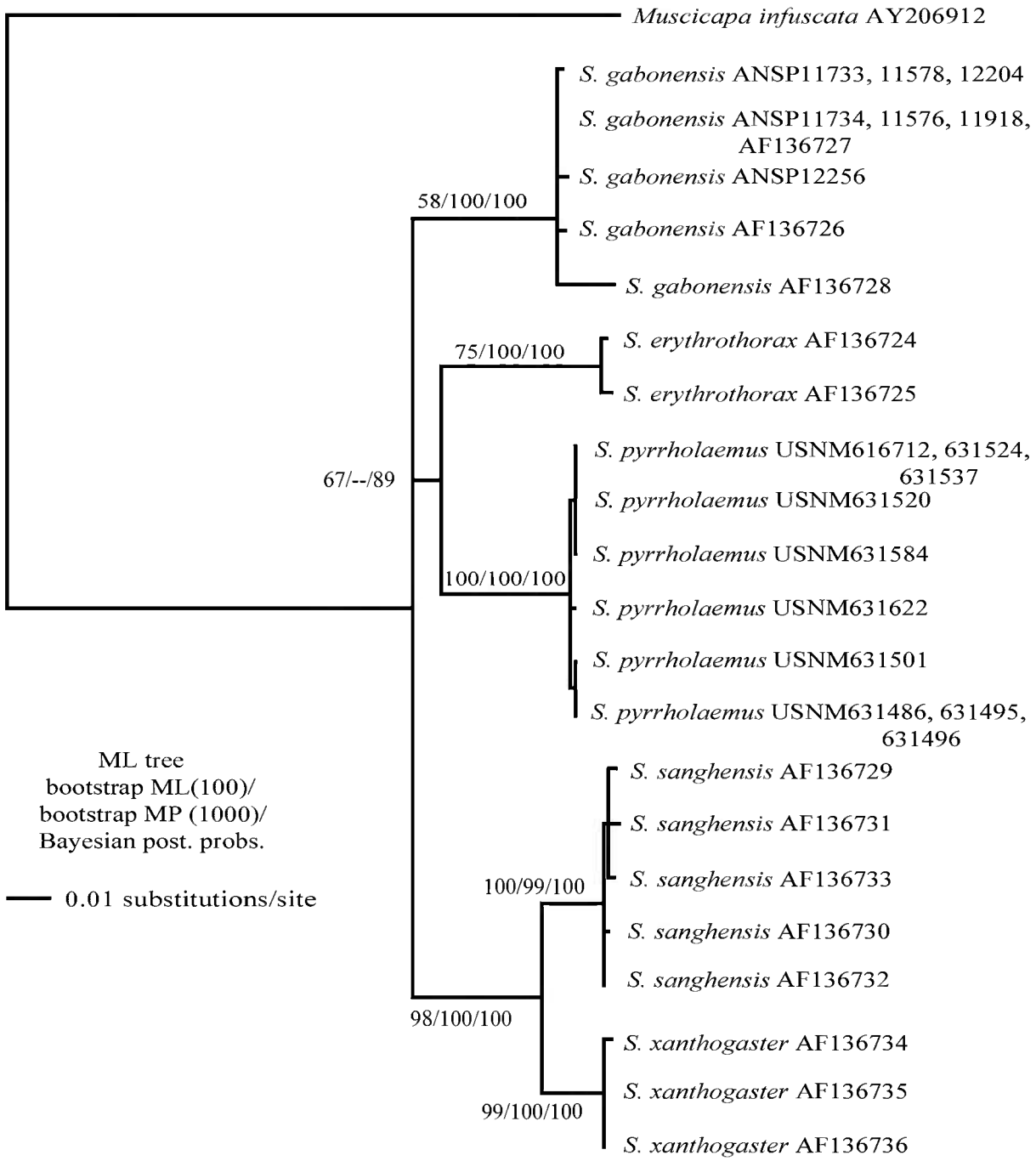

FIGURE 5. Phylogram of *Stiphrornis* species and outgroup showing phylogenetic relationship of *S. pyrrholaemus* to closely related species. Tree shown is from a maximum-likelihood heuristic search, although maximum-parsimony and Bayesian analysis gave same topology. Samples with names starting with AF are GenBank accession numbers from the work of Beresford and Cracraft (1999).

Although *S. pyrrholaemus* is clearly differentiated from all other *Stiphrornis*, the precise relationships among the species in this genus are uncertain. Limited bootstrap support indicates that the *erythrothorax–pyrrholaemus* clade cannot be reliably distinguished from the other species, particularly *S. gabonensis* whose placement is ambiguous. The topology of our tree is similar to that of the maximum parsimony tree of Beresford and Cracraft (1999), where *S. sanghensis* and *S. xanthogaster* are closely related sister species. We have no indication of hybridization between species but the possibility occurs due to the overlap in *Stiphrornis* species ranges. Detailed breeding studies of these species have yet to be conducted but should be informative regarding interspecific interactions and the potential for hybridization. Additional sequencing of other mitochondrial and nuclear regions should better resolve the relationships within the genus and identify potential mixing in hybrid zones, if they exist.

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