



NOTES ON GEOGRAPHIC DISTRIBUTION

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## First record of *Chiroderma improvisum* Baker & Genoways, 1976 (Chiroptera: Phyllostomidae) from Saint Kitts, Lesser Antilles

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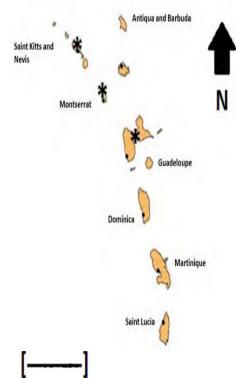
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**Abstract:** Chiroderma improvisum is a rare bat previously known only on the Caribbean Islands of Guadeloupe and Montserrat. We report the first recorded capture of *C. improvisum* on the island of Saint Kitts, 80 km northwest of Montserrat. Cytochrome b (cytB) gene analysis of the single captured specimen confirmed the identity of the bat as *C. improvisum*; however, there is enough difference to indicate some population divergence, and possibly differentiation at the subspecific level among islands. We also report the first records of an ectoparasite, *Periglischrus iheringi* (Acarina: Spinturnicidae), from this bat.

**Key words:** Chiroptera; Phyllostomidae; Caribbean; Lesser Antilles; vulnerable species; *Periglischrus iheringi*, Spinturnicidae

One of the largest and rarest of bats in the Caribbean, Chiroderma improvisum Baker & Genoways, 1976, is known only from a handful of infrequent encounters on two islands located in the Lesser Antilles of the eastern Caribbean: Guadeloupe, where single bats were captured in 1974, 2000, and 2007, and Montserrat, from which specimens were captured in 1978, 1984, and 2005 (Figure 1) (Baker and Genoways 1976; Baker et al. 1978; Jones and Baker 1979; Larsen et al. 2007). Chiroderma improvisum was not reported from Saint Kitts in the catalog of bats from the island by Pedersen et al. (2005). Conservation and population status of this species are poorly understood and the bat is listed as vulnerable, due to restricted geographic range and habitat conversion by the increasing human population on these islands (Dávalos and Rodriguez 2008). Parasites and pathogens are unknown from this species. We report the capture of a single specimen of *C. improvisum* from the nation of Saint Kitts and Nevis, in the northern Lesser Antilles. Saint Kitts Island consists of a composite volcanic island with some limestone uplift formations located 180 km northwest of Guadeloupe and 80 km northwest of Montserrat (Davis 1924).

The capture of this individual bat was incidental to a project conducted with Ross University School of Veterinary Medicine, which was examining bats on Saint Kitts for internal and external parasites. The project was approved by the Institutional Animal Care



**Figure 1**. Islands of the Eastern Caribbean from Saint Kitts and Nevis to Saint Lucia. Islands with collection records of *Chiroderma improvisum* marked with an asterisk. Coordinates of historical collections were not reported in the literature. The Saint Kitts and Nevis collection was at 17°19.222' N, 062°43.615' W using datum WGS84. Scale bar is 150 km.

and Use Committee of Ross University. There is little exposed fresh water on Saint Kitts, so we sought out streams, ponds, and swimming pools for mist netting sites. During November to December 2010, we captured and examined 92 bats (51 Artibeus jamaicensis Leach, 1921, 20 Ardops nichollsi (Thomas, 1891), 13 Molossus molossus (Pallas, 1766), 7 Brachyphylla cavernarum Gray, 1834, and 1 Chiroderma improvisum). Bats were identified to species, with sex, reproductive status, total body weight, and morphometric measurements recorded. Body measurements were made with calipers. We were not approved to kill C. improvisum as it is included on the IUCN Red List of Threatened Species, and the individual was photographed and released after blood and ectoparasite samples were taken.

Additionally, a thin blood film was prepared, stained with the CAMCO (Cambridge Diagnostic Products, Inc., Fort Lauderdale, FL) Stain Pak, and examined for the presence of blood parasites. Culture for bacteria in the genus *Bartonella* spp. was performed, using 50  $\mu$ L of blood plated onto BHI/Sheep Blood Agar (Remel Products, Lenexa, KS), as previously described (Kosoy et al. 2010). DNA from blood was tested for *Bartonella* spp. using a sensitive real-time PCR assay (Reeves et al. 2007). *Bartonella* spp. are known to infect several species of tropical bats (Kosoy et al. 2010).

We obtained a 0.3 mL blood sample from the brachial vein of the *C. improvisum*. A 200-µL aliquot was used for DNA extraction, using a DNeasy kit (Qiagen, Valencia,





**Figure 2.** Photographs of the adult female of *Chiroderma improvisum* captured on Saint Kitts, 28 November 2010.

CA). PCR primers StBr-1F (5'-ATG ACC AAC ATT CGA AAA ACI CAC C) and StBr-533R (5'-AAT CGG GYG AGR GTT GCT TTG TCT) were designed to amplify the 5' end of the cytochrome b oxidase mitochondrial gene, based upon an alignment generated from Lesser Antillean species of bats (A. nichollsi, Genbank #AY572337; A. jamaicensis, GQ861667; C. improvisum, L28938; and Stenoderma rufum, AY604432). The PCR amplicon was sequenced by a commercial laboratory (Davis Sequencing, Davis, CA), yielding a 484 bp sequence after primers were trimmed (GenBank accession #JQ915203). Phylogenetic analysis using neighbor joining was performed using PAUP 2.0 beta software (Swofford 2003).

The adult female (Figure 2) of C. improvisum was netted near a house (17°19.222' N, 062°43.615' W using datum WGS84) on 28 November 2010. Our collection is the first with georeferenced data in the literature (Figure 1). External measurements of forearm length 59 mm, tibia length 27 mm and mass of 4.7 g conform to what is known for the species (Table 1). It did not appear to be lactating or pregnant, as was typical of other species that we examined during late November. Pelage was dark grey/brown. There was some frosting throughout but more so ventrally. The dorsal pelage was purer brown and a thin white stripe was clearly evident. Chiroderma improvisum is the largest known Chiroderma with Chiroderma doriae being the only other similarsized species (Baker and Genoways 1976). Our specimen was within the morphometric range of C. improvisum from Guadeloupe and Montserrat (Baker and Genoways 1976; Jones and Baker 1979) and larger than the other Chiroderma species (Table 1). In addition, we used molecular tools to confirm the species identification.

Two parasitic mites in the family Spinturnicidae mites were collected, slide mounted, and identified using a stereoscopic microscope using keys by Furman (1966) and Morales-Malacara (2001). The specimens were deposited as vouchers GM/S<P>007 and GM/S<P>044 at Tomsk State University, Siberia, Russian Federation.

The cytochrome B gene has been sequenced for all named forms of *Chiroderma* (available on GenBank). Blood from the *C. improvisum* specimen was used for genetic analysis. The cytochrome B gene fragment was 98.6% (477/484 bp) identical to that from a *C.* 

**Table 1**. Summary of morphological characters of *Chiroderma improvisum* comparing the St. Kitts specimen to those reported for all other *Chiroderma spp.* by Jones and Genoways (1976). All measurements are given in millimeters (mm).

Name	Pelage color	Forearm length	Head and body length
Chiroderma improvisum <sup>a</sup>	brown with dorsal white stripe	59	70
Chiroderma improvisum <sup>b</sup>	brown with dorsal white stripe	57.3–57.5 ( <i>n</i> = 2)	87
Chiroderma villosum	light brown dorsal stripe present or absent	46.6-47.3	64–73
Chiroderma trinitatum	light brown with dorsal white stripe	38.9-41.8	55-69
Chiroderma salvini	dark brown with dorsal white stripe	43.7-47.5	62-72
Chiroderma doriae	greyish brown with white stripe/grey	51.0-55.5	70–80

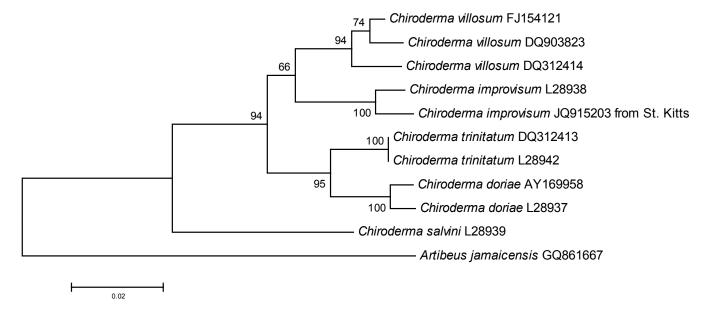
<sup>&</sup>lt;sup>a</sup> Specimen from Saint Kitts.

 $<sup>^{\</sup>rm b}$  Specimens from Guadeloupe and Montserrat (n=2). Both specimens were female.

improvisum museum specimen collected on Monserrat (Genbank #L28938; Baker 1994). Phylogenetic analysis was then used to compare the DNA sequence from the *Chiroderma* specimen from Saint Kitts with other available sequences from *Chiroderma* species (Figure 3); this analysis confirms that the specimen from Saint Kitts is *C. improvisum*, although it is slightly divergent from the previously accessioned sequence.

Male and female mites were collected from the *C. improvisum* from Saint Kitts. Both mites were identified as *Periglischrus iheringi*. These parasitic mites are found primarily on the wing membranes of Phyllostomidae (Furman 1966). No blood-borne parasites or *Bartonella* 

and size of the bat is similar to *C. improvisum* found on Guadeloupe and Montserrat (Jones and Baker 1980), but a possible darker coloration and 1.4% variation in the genetic analysis could indicate separation from the previously identified populations. However, both our sample and the diversity of reference sequences in GenBank are quite limited. In our phylogenetic analysis, similar within-species divergence was noted between published sequences from specimens of *Chiroderma villosum* and *Chiroderma doriae*. Subspeciation of bats located on different islands of the Lesser Antilles has been reported for other endemic bat species, including *A. nichollsi* and *B. cavernarum* (Jones and Genoways



**Figure 3**. Neighbor-joining tree comparing the 484-bp sequence of the cytochrome B gene with other published *Chiroderma* spp. sequences (GenBank accession numbers indicated). *Chiroderma improvisum* (L28938) was collected on Montserrat (Baker et al. 1994). *Artibeus jamaicensis* was included as an outgroup. Bootstrap replicates (*n*=1000) were performed, and bootstrap support is displayed as a precentage for each node. The scale bar indicates the expected number of nucleotide substitutions per site.

bacteria were detected in this bat, either by examination of a stained blood film, culture to isolate *Bartonella* spp., or by sensitive real-time PCR testing for *Bartonella*.

The *C. improvisum* was captured approximately 3 hours after sunset on 28 November 2010, in a  $12\,\mathrm{m} \times 2.6\,\mathrm{m}$  mist net set near an abandoned swimming pool that had collected 1.5 m of rainwater. Bats had to fly below ground level to get to the water. The water was clear but contained a small amount of debris and a large number of larval amphibians (tadpoles). Most of the trees on the island were not bearing fruit, but a large fruiting fig tree located 50 m from the pool was attracting large numbers of fruit bats. The net was intentionally placed between these the pool and tree.

External measurements, qualitative pelage characters, and mtDNA analysis confirm the identity of the Saint Kitts as *Chiroderma improvisum*. The external appearance

1973; Swanepoel and Genoways 1983). Additional DNA samples from *C. improvisum* from all three islands would be required to address this possibility; however, this bat is rarely captured, and all captures reported to date have included single specimens that were either released or deposited in museums.

The parasitic mite *P. iheringi* is a common ectoparasite of phyllostomids. It has not been reported on *C. improvisum*. *Periglischrus iheringi* is primarily found on wing membranes and probably feeds on both the host and other ectoparasites. It was reported on other species of *Chiroderma* by Smith (2012). *Periglischrus iheringi* was also collected from the other Phyllostomidae on Saint Kitts. It is not known to transmit pathogens to bats but has been associated with bacteria in the family Anaplasmataceae bacteria that might be an endosymbiont or possibly a pathogen (Reeves et al. 2006).

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**Author contributions:** JB, AL, and JD conducted fieldwork in Saint Kitts and caught the bat. Ectoparasites were identified by WR and MO. Pathogen screening was done by AL. Drafts of the manuscript were reviewed and parts were written by all authors.

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