Cryptic diversity and range extension in the big-eyed bat genus *Chiroderma* (Chiroptera, Phyllostomidae)

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Abstract
Since the last systematic review of *Chiroderma* (big-eyed bats) more than two decades ago, we report on biodiversity surveys that expand the distribution and species diversity of this Neotropical genus. The Caribbean endemic species *Chiroderma improvisum* is documented for the first time from Nevis in the northern Lesser Antilles. A broader geographic sampling for a molecular analysis identifies a paraphyletic relationship in *Chiroderma trinitatum* with respect to *Chiroderma doriae*. Cis-Andean populations of *C. trinitatum* are most closely related to the morphologically distinctive and allopatrically distributed *C. doriae* in the Cerrado and Atlantic Forest of Brazil and Paraguay. The sister taxon to this grouping includes trans-Andean populations of *C. trinitatum*, which we recommend to elevate to species status as *C. gorgasi*. This is an example of a cryptic species because *C. gorgasi* was previously considered morphologically similar to *C. trinitatum*, but more detailed examination revealed that it lacks a posterolabial accessory cusp on the lower second premolar and has a narrower breadth of the braincase. We provide an amended description of *Chiroderma gorgasi*.

Keywords
*Chiroderma gorgasi, Chiroderma improvisum*, cryptic species, cytochrome c oxidase subunit 1, Lesser Antilles
**Introduction**

Cryptic species, phenotypically similar organisms that are classified as a single species but are genetically divergent lineages, are being discovered at a greater rate due to the increasing prevalence of molecular methods, such as DNA barcoding (e.g., Hebert et al. 2004). It has been estimated that Neotropical mammalian biodiversity is underestimated by one-third (Lim 2012). At typical lowland tropical forest sites, bats comprise the majority of mammal species diversity (Voss and Emmons 1996), so more species are expected to be recognized in this group as traditional taxonomic hypotheses are tested by genetic techniques. In addition, new surveying methods such as the use of triple-high netting systems to catch higher flying aerial insectivorous bats, and harp traps to target species that may be able to better detect mist nets, is decreasing the sampling bias associated with traditional mist nets set just above ground level.

The big-eyed bats in the genus *Chiroderma* Peters (Phyllostomidae) are characterized by greatly reduced nasal bones in the skull and a combination of external features including a white dorsal stripe that does not extend onto the head; legs and interfemoral membrane conspicuously hairy; and relatively large eyes (Straney 1984; Gardner 2008). The genus currently comprises six species (Simmons 2005, Taddei and Lim 2010): *C. doriae* Thomas, 1891 occurs in central-eastern Brazil and Paraguay; *C. improvisum* Baker & Genoways, 1976 is endemic to the Lesser Antillean islands of Guadeloupe, Montserrat, and Saint Kitts (Beck et al. 2016); *C. salvini* Dobson, 1878 is found from Mexico to Bolivia (recent records from Brazil are misidentifications of *C. villosum* Peters, 1860 – see Brandão et al. 2019); *C. trinitatum* Goodwin, 1958 is distributed from Honduras (Turcios-Casco et al. 2020) and Costa Rica to Amazonian Brazil and Trinidad; *C. villosum* ranges from Mexico to southeastern Brazil and Trinidad; and *C. vizottoi* Taddei & Lim, 2010 is found only in northeastern Brazil.

The systematics of *Chiroderma* was last reviewed by Baker et al. (1994) based on a phylogenetic study of the mitochondrial DNA cytochrome *b* (Cytb) gene; however, each of the five species known at the time was represented by a single specimen. With broader geographic coverage, we re-assess the distributional range, genetic diversity, and morphological differences in the genus.

**Material and methods**

**Fieldwork**

We conducted a survey of bats on the Caribbean island of Nevis from 24–29 April 2016. Live traps used included a harp trap and 6 m or 12 m mist nets set singly in the forest understory or on a triple-high telescoping pole system. Traps were regularly monitored for the first 2–3 hours after sunset when bat activity is the highest after they leave their roosts to feed. Individuals not kept as part of the representa-
ative collection documenting the species diversity were released at point of capture. A combined scientific research and export permit (F002) was issued through the authority of the Nevis Historical and Conservation Society. An Animal Use Protocol (2016-01) was obtained from the Royal Ontario Museum Animal Care Committee. An import permit (#2016-02101-4) was authorized by the Canadian Food Inspection Agency. Use of wild mammals followed the guidelines of the American Society of Mammalogists (Sikes et al. 2016).

**Molecular analyses**

The cytochrome c oxidase subunit 1 (CO1) gene is the best represented molecular marker for *Chiroderma* on the genetic sequence database GenBank ([www.ncbi.nih.gov/genbank](http://www.ncbi.nih.gov/genbank)). There are 117 samples from nine countries in Central and South America (Brazil, Ecuador, El Salvador, French Guiana, Guatemala, Guyana, Mexico, Panama, and Suriname). We add 26 new sequences to bring the sample size to 143 sequences representing 12 countries in the Neotropics, including Venezuela, Peru, and Nevis, and five species in the genus (Appendix 1). There are no tissue samples or nucleotide sequences on GenBank of any genes for the recently described *Chiroderma vizottoi* (Taddei and Lim 2010). Outgroup taxa were other genera in the subtribe Vampyressina Baker et al., 2016 (*Platyrhinus incarum* Thomas, 1912 and *Uroderma bilobatum* Peters, 1866) of the New World leaf-nosed bats, for direct comparison to Baker et al. (1994) in their analysis of Cytb. Alternative phylogenetic relationships within the subtribe are given by Baker et al. (2016) and Rojas et al. (2016). We also analyzed Cytb, but there are only 11 sequences on GenBank, although we did add one new sequence of *Chiroderma trinitatum gorgasi* from Panama (Appendix 2).

Molecular methods for new sequences of CO1 follow the protocol for DNA extraction, PCR amplification, and automated nucleotide sequencing outlined in Lim (2017). For Cytb, extraction, amplification, and sequencing followed Lim et al. (2008). Base calls were confirmed with bidirectional sequences and aligned using Sequencher version 4.8 (Gene Code Corporation, Ann Arbor, Michigan). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 6 (Tamura et al. 2013). For a robust comparison of phylogeny, we used parsimony as a method that minimizes evolutionary change without an explicit model of evolution and maximum likelihood as a probabilistic method with an explicit model of evolution. Maximum parsimony used the subtree pruning regrafting inference method with 500 bootstrap replicates to test branch supports. Maximum likelihood used the Tamura 3-parameter substitution model and gamma distributed rates with invariant sites for COI as determined by the best fit test. For Cytb, the Tamura Nei model and gamma rates were the best fit. Tree inference used nearest neighbor interchange heuristic inference with 500 bootstrap replicates. Genetic distances were calculated with the Tamura 3-parameter model with gamma distributed rates among sites for the larger COI dataset.
Morphological analyses

Morphological and morphometric comparisons included 138 specimens from five species of *Chiroderma*, including two *C. improvisum*, four *C. doriae*, seven *C. salvini*, 58 *C. trinitatum*, and 66 *C. villosum* (Appendix 3). We also analyzed the holotypes of *C. trinitatum gorgasi* Handley, 1960 and *C. trinitatum trinitatum* Goodwin, 1958, but did not have specimens of the most recently described *C. vizottoi*. Only adults (defined as having closed cranial sutures and complete epiphyseal ossification of metacarpal and phalanx joints) of both sexes were examined. Specimens are deposited in the following institutions; Royal Ontario Museum (ROM, Toronto, Canada); National Museum of Natural History (USNM, Washington, DC, USA); American Museum of Natural History (New York, USA); Texas Tech University (Lubbock, USA); and Field Museum of Natural History (Chicago, USA).

Measurements defined below were taken with digital calipers accurate to 0.01 mm following the descriptions of Handley (1960): forearm length (FA); greatest length of skull (GSL); interorbital width (IOW); postorbital width (POW); braincase width (BCW); condyloincisive length (CIL); zygomatic breadth (ZB); width across upper molars (M-M); width across upper canines (C-C); and length of maxillary toothrow (C-M). An analysis of variance (ANOVA) for each measurement and a multivariate analysis of variance (MANOVA) were performed to examine the significance of morphometric divergence among species of *Chiroderma*. The level of significance was *p* = 0.05 for all statistical tests. The homoscedasticity of each variable was tested using Bartlett’s test with the R package mvoutliers. Statistical analyzes were performed using R 3.1.0 (R Core Team 2005) and PAST 2.17. Variables were log-transformed and a correlation matrix was used in a Principal Components Analysis (PCA) to assess phenetic differences in multivariate morphological space.

Results

We report the first occurrence of *Chiroderma improvisum* (Fig. 1) from Nevis in the northern Leeward Islands of the Lesser Antilles in the Caribbean. An adult male was caught at Barnes Ghaut on April 28, 2016, in a harp trap set across a dry ravine in forest bisected by a road and surrounded by residential homes (Fig. 2). Other equipment deployed included 6 m mist nets set on a triple-high telescoping pole system, a single 6 m mist net, and a single 12 m mist net from 1900–2100 h. In addition to the new distributional record for the island, one *Ardops nichollsi*, one *Noctilio leporinus*, and 12 *Artibeus jamaicensis* were captured.

Molecular analyses

For COI, the 657 basepairs (bp) at the 5’ end were available for most (82%) of the specimens analyzed. The complete 1140 bp of Cytb were available, including the newly generated sequence, for most (75%) of the specimens analyzed. The topology of the *Chiroderma* COI maximum likelihood tree identified six primary terminal clades with (1) *C. salvini* as
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Figure 1. First record of the big-eyed bat *Chiroderma improvisum* from Nevis (ROM 126002).

Figure 2. Collecting locality of the first record of the big-eyed bat *Chiroderma improvisum* from Nevis caught in a harp trap on April 28, 2016. The habitat is a dry ravine within forest that is bisected by a road in the residential area of Barnes Ghaut.
sister species to all other taxa; (2) *C. improvisum* and (3) *C. villosum* as sister species; and (4) *C. doriae* sister to (5) *C. trinitatum trinitatum* with (6) *C. trinitatum gorgasi* sister to these taxa (Fig. 3; Suppl. material 1: Fig. S1). These phylogenetic relationships were supported by bootstrap values ≥85 and were congruent with the maximum parsimony tree (Suppl. material 2: Fig. S2), which had bootstrap values ≥73. Not surprisingly for linked mtDNA loci, the same interspecific topology was recovered by the smaller Cytb dataset analyzed by maximum likelihood (Suppl. material 3: Fig. S3) and maximum parsimony (Suppl. material 4: Fig. S4), except for lower bootstrap supports. The unexpected result was the paraphyly of *C. trinitatum* in relation to *C. doriae*. The sister-group relationship of *C. t. trinitatum* and *C. doriae* was well supported by values ≥73 in all molecular analyses.

Interspecific genetic distances of the larger COI dataset ranged from 11.3% between *C. doriae* and *C. salvini* to 2.5% between *C. doriae* and *C. t. trinitatum* (Table 1). The sequence divergence between *C. t. trinitatum* and *C. t. gorgasi* was 3.9%. Intraspecific distances were 1% within *C. villosum*, 0.9% within *C. t. trinitatum*, and 0.2% within *C. doriae*, but three taxa were represented by only one sample.

**Morphological analyses**

Cranial and body measurements for the six taxa of *Chiroderma* identified in the molecular analyses are shown in Table 2. *Chiroderma trinitatum gorgasi* and *C. trinitatum trinitatum* are the smallest members of the genus, whereas *C. improvisum* is the largest for most measurements. In the PCA, there are three main groups of species (Fig. 4). The
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Table 1. Genetic divergence of cytochrome c oxidase subunit 1 for the big-eyed bat Chiroderma and outgroup taxa Uroderma and Platyrrhinus. Interspecific distances shown in the lower left matrix; intraspecific distances shown in bold in the diagonal.

|                  | (1)  | (2)  | (3)  | (4)  | (5)  | (6)  | (7)  | (8)  |
|------------------|------|------|------|------|------|------|------|------|
| U. bilobatum (1) | –    |      |      |      |      |      |      |      |
| P. incarum (2)   | 0.203| –    |      |      |      |      |      |      |
| C. villosum (3)  | 0.223| 0.178| 0.010|      |      |      |      |      |
| C. improvisum (4)| 0.231| 0.194| 0.047| –    |      |      |      |      |
| C. t. trinitatum (5)| 0.222| 0.184| 0.067| 0.075| 0.009|      |      |      |
| C. salvini (6)   | 0.205| 0.185| 0.093| 0.101| 0.110| –    |      |      |
| C. t. gorgasi (7) | 0.195| 0.149| 0.059| 0.070| 0.039| 0.101| –    |      |
| C. doriae (8)    | 0.213| 0.173| 0.066| 0.077| 0.025| 0.113| 0.039| 0.002|

Table 2. Cranial and body measurements of six taxa of the big-eyed bat Chiroderma. See Material and methods for variable abbreviations.

|                  | C. trinitatum gorgasi (N = 11) | C. trinitatum trinitatum (N = 47) | C. villosum (N = 66) | C. salvini (N = 6) | C. doriae (N = 4) | C. improvisum (N = 2) |
|------------------|-------------------------------|-----------------------------------|----------------------|-------------------|-------------------|-----------------------|
| FA               | 37.7 (37.0–40.5)              | 38.9 (37.1–42.6)                  | 47.9 (44.6–51.0)     | 49.6 (49.3–50.0)  | 54 (53.0–55.0)    | 58.2 (56.2–60)        |
| GLS              | 21.2 (20.6–21.7)              | 21.1 (20.0–22.7)                  | 24.5 (23.2–25.7)     | 26.1 (24.2–26.5)  | 28.2 (27.6–28.9)  | 29.3 (28.7–29.9)      |
| CLI              | 17.3 (16.3–18.1)              | 17.4 (16.1–18.8)                  | 20.3 (18.7–21.58)    | 21.6 (21.5–22.3)  | 23.9 (23.1–24.5)  | 26.3 (25.3–27.8)      |
| ZB               | 13.0 (12.5–13.5)              | 12.9 (11.7–14.2)                  | 15.5 (14.4–16.7)     | 16.2 (15.8–16.7)  | 17.84 (17.7–18.1) | 18.75 (18.5–19)       |
| POW              | 5.3 (4.9–5.6)                 | 5.3 (5.8–5.8)                     | 5.9 (5.3–6.3)        | 6.2 (5.9–6.3)     | 6.4 (6.1–6.6)     | 6.6 (6.5–6.6)         |
| IOW              | 5.6 (5.2–5.9)                 | 5.5 (5.0–6.2)                     | 6.0 (5.5–6.8)        | 6.8 (6.1–7.3)     | 7.6 (7.1–7.8)     | 7.4 (7.4–7.4)         |
| BCW              | 9.4 (8.9–9.8)                 | 9.6 (9.2–10.4)                    | 10.7 (10.1–11.5)     | 11.21 (11.0–11.5) | 11.9 (11.3–12.1)  | 12.0 (11.5–12.5)      |
| C-M              | 7.0 (6.5–7.3)                 | 7.1 (6.7–7.8)                     | 8.7 (8.1–9.4)        | 9.4 (9.1–9.4)     | 10.3 (10.0–11.1)  | 11.0 (10.9–11.1)      |
| M-M              | 9.6 (9.2–10.0)                | 9.5 (8.7–10.3)                    | 11.3 (10.3–12.4)     | 12.0 (11.5–12.3)  | 13.3 (13.0–13.7)  | 13.8 (13.6–13.9)      |
| C-C              | 4.7 (4.4–5.0)                 | 4.6 (4.1–5.0)                     | 5.8 (5.3–6.3)        | 6.1 (6.0–6.2)     | 6.5 (6.3–6.8)     | 7.4 (7.4–7.4)         |

Figure 4. Principal Component Analysis (PCA) carried out using the correlation matrix of ten measurements for six taxa of the big-eyed bat Chiroderma. C. trinitatum gorgasi (■), C. trinitatum trinitatum (□), C. salvini (×), C. villosum (+), C. doriae (●), and C. improvisum (◊).
Table 3. Eigenvalue and loadings for the first and second components in the Principal Component Analysis (PCA) of big-eyed bats Chiroderma. See Material and methods for variable abbreviations.

|         | PC1     | PC2     |
|---------|---------|---------|
| Eigenvalue | 1.57    | 0.53    |
| % Variance  | 91.4    | 3.11    |
| IOW       | 0.25    | 0.44    |
| POW       | 0.21    | 0.40    |
| C-M       | 0.34    | -0.23   |
| GSL       | 0.31    | -0.05   |
| BCW       | 0.34    | 0.35    |
| CIL       | 0.34    | 0.07    |
| C-C       | 0.36    | -0.23   |
| M-M       | 0.36    | -0.14   |
| ZB        | 0.37    | -0.12   |
| FA        | 0.42    | 0.32    |

The first group is formed by the smaller taxa *C. t. gorgasi* and *C. t. trinitatum*. The second group has species with medium size, *C. villosum* and *C. salvini*, and the third group is formed by the largest species of the genus, *C. doriae* and *C. improvisum*. The first and second principal components (PC1 and PC2) explained 94.5% of the total variation. PC1 shows a pattern in general size variation and is explained mostly by C-M, C-C, and FA. PC2 has positive loadings for most measurements, especially IOW, with the exception of C-M, C-C, M-M, and ZB that have negative loadings (Table 3). All the species seem to occupy the entire range of PC2, indicating that the contrast among measurements is negligible and it is not responsible for the separation of groups.

All variables had \( p > 0.05 \) for Bartlett’s test of homoscedasticity, indicating constant variances (\( p \) values: FA = 0.06, GSL = 0.25, IOW = 0.59, POW = 0.31, BCW = 0.06, CIL = 0.45, ZB = 0.08, M-M = 0.32, C-C = 0.08, and C-M = 0.06). The MANOVA and the ANOVA demonstrated that *C. t. trinitatum* and *C. t. gorgasi* are significantly different from the other taxa of *Chiroderma* (\( P < 0.001 \)) for all measured variables (Appendix 3). By contrast, *C. t. gorgasi* and *C. t. trinitatum* are not significantly different from each other (\( p = 0.16; F = 56.0 \)). However, the ANOVA showed that one measurement, BCW (\( p = 0.01; F = 62.0 \)), was significantly larger for *C. t. trinitatum* than for *C. t. gorgasi*. All other cranial measurements had smaller mean values for *C. t. trinitatum* than for *C. t. gorgasi*.

Although similar in size, *C. t. trinitatum* has a more robust breadth of the braincase than *C. t. gorgasi*. *Chiroderma t. trinitatum* also has an accessory cusp on the second lower premolar, which is absent in *C. t. gorgasi* (Fig. 5). In the genetic analyses, *C. t. trinitatum* is well supported as the sister species to *C. doriae* and does not share a most recent common ancestor with *C. t. gorgasi*. We consider this as a previous example of a cryptic species and therefore now recognize *C. gorgasi* as a distinct species from *C. trinitatum*. Sáez and Lozano (2005: 111) considered cryptic species to be “groups of organisms that are morphologically indistinguishable from each other, yet found to belong to different evolutionary lineages”. They also stated that “after detailed comparisons of morphological and non-morphological features, we can often establish key
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Figure 5. Lateral view of the second lower premolar on the right mandible of A Chiroderma gorgasi and B C. trinitatum. The arrow points to the accessory cusp that is absent in C. gorgasi and present in C. trinitatum. But note the variation in cusp formation in C. trinitatum.

morphological characters for their identification. In those cases, we can then refer to pseudo-cryptic or pseudo-sibling species”. Because Handley’s original description was qualitative and univariate, we offer an amended description of this taxon.

Taxonomic account

Chiroderma gorgasi Handley, 1960

Chiroderma gorgasi Handley, 1960:464
Chiroderma trinitatum gorgasi Barriga-Bonilla, 1965:246

Material examined. Holotype. – USNM 309903 (Field number COH 5436), adult male with skin, skull and partial skeleton. Collected on March 6, 1959, by C. O. Handley, Jr, and B. R. Feinstein in Tacarcuna Village (8°05’N, 77°17’W), 3200 feet [975 meters], Río Pucro, Darién, Panama.

Geographic distribution. Chiroderma gorgasi is distributed west of the Andes in northwestern Ecuador (Albuja 1989), western Colombia (Gardner 2008), Panama
Figure 6. Geographic distribution of *Chiroderma gorgasi* (▲) and *C. trinitatum* (●) localities analyzed in our study (See Appendix 3) (●) Represents marginal localities reported for *C. trinitatum* and (▲) *C. gorgasi* reported by previous papers (Handley 1967; Pine et al. 1970; Ojasti and Linares 1971; Gardner 1976; Albuja 1989; Timm and LaVal 1998; Lim and Engstrom 2001; Genoways et al. 1981; Webster and Fugler 1984; Anderson 1997; Ochoa et al. 1988; Simmons and Voss 1998; Gardner 2008).

(Handley 1960), Costa Rica (LaVal and Rodríguez-Herrera 2002), and Honduras (Turcios-Casco et al. 2020) (Fig. 6).

**Description.** *Chiroderma gorgasi* is a small species of *Chiroderma* (FA 37.0–40.5; GLS 20.2–22.5) that is similar in size to *C. trinitatum* (sensu stricto) (Table 2). Overall, the dorsal pelage is tricolor varying from light to dark brown (Fig. 7). The dorsal hairs have a dark brown band at the base, a buff coloration in the middle, and brown tips. A white medial stripe extends from the interscapular region to the base of the rump. Proximal two-thirds of forearm hairy. Basal third of uropatagium hairy. Conspicuous white facial stripes extend from the noseleaf to the inner base of the ears, and from the posterior part of the upper lip to the base of the ears. The uropatagium is medium brown. The skull has an elongated braincase with an undeveloped sagittal and lambdoidal crest. The nasal aperture is short, not extending beyond the second premolar. The occipital is rounded in posterior view. The upper incisors are thin and elongated with parallel or convergent tips, which may or may not touch apically. The second lower premolar lacks a third cusp (Fig 5). The postorbital processes are undeveloped and rounded (Fig. 8).

**Comparisons.** *Chiroderma gorgasi* is morphologically very similar to *C. trinitatum*. Both species have a small cranial and body size for the genus (Table 2, Fig. 4), an
Figure 7. Dorsal view of the skin of the holotype of Chiroderma gorgasi (USNM 309903).
undeveloped sagittal and lambdoidal crest, a rounded occipital complex, a short nasal aperture, and undeveloped supraorbital region. However, *C. trinitatum* has a third posterior cusp on the second lower premolar, which is absent in *C. gorgasi* (Fig. 5). This cusp in *C. trinitatum* may vary from very pointed and developed to rounded and less marked, but is always present. In addition, *C. gorgasi* tends to have a broader braincase (Table 4) and a flatter supraorbital region, which tends to be deeper in *C. trinitatum*.

*C. gorgasi* is easily distinguished from other species of the genus by its smaller cranial and body size (Table 2). *C. villosum* shares with *C. gorgasi* an elongated braincase, rounded occipital region in dorsal view, and absence of a third cusp on the second lower premolar. However, *C. gorgasi* has an undeveloped postorbital processes, a short nasal aperture, and conspicuous white stripes on the face and back, whereas *C. villosum* has a very developed and pointed postorbital processes, a long nasal ap-
Table 4. ANOVA comparing Chiroderma trinitatum trinitatum to C. doriae, C. improvisum, C. villosum, C. salvini, and C. trinitatum gorgasi. * indicates significantly distinct variables ($p \leq 5\%$).

| Variables | C. doriae | C. improvisum | C. villosum | C. salvini | C. t. gorgasi |
|-----------|-----------|---------------|-------------|------------|--------------|
| GSL       | < 2.2e-16*| < 2.2e-16*    | < 2.2e-16*  | < 2.2e-16* | 0.1541       |
| CI        | < 2.2e-16*| < 2.2e-16*    | < 2.2e-16*  | < 2.2e-16* | 0.4423       |
| ZB        | < 2.2e-16*| < 2.2e-16*    | < 2.2e-16*  | < 2.2e-16* | 0.3094       |
| POW       | < 2.2e-16*| < 2.2e-16*    | < 2.2e-16*  | 1.061e-12* | 0.6747       |
| IOW       | < 2.2e-16*| < 2.2e-16*    | 2.366e-14*  | 1.355e-14* | 0.6272       |
| BWC       | < 2.2e-16*| < 2.2e-16*    | < 2.2e-16*  | < 2.2e-16* | 0.0339*      |
| M-C       | < 2.2e-16*| < 2.2e-16*    | < 2.2e-16*  | < 2.2e-16* | 0.5257       |
| B-M       | < 2.2e-16*| < 2.2e-16*    | < 2.2e-16*  | < 2.2e-16* | 0.1444       |
| B-C       | < 2.2e-16*| < 2.2e-16*    | < 2.2e-16*  | < 2.2e-16* | 0.1251       |
| FA        | < 2.2e-16*| < 2.2e-16*    | < 2.2e-16*  | < 2.2e-16* | 0.1753       |

Chiroderma trinitatum trinitatum to C. doriae, C. improvisum, C. villosum, C. salvini, and C. trinitatum gorgasi. * indicates significantly distinct variables ($p \leq 5\%$).

Discussion

The only big-eyed bat species occurring in the Caribbean is Chiroderma improvisum, which until recently was known from Guadeloupe (Baker and Genoways 1976) and Montserrat (Jones and Baker 1979; Pierson et al. 1986) by six individuals (Larsen et al. 2007). Subsequently, it was caught on Saint Kitts by Beck et al. (2016) and we are the first to report its occurrence on Nevis. Although this species has been sporadically documented since its discovery, the distribution has broadened in the northern Lesser Antilles but this may be ephemeral depending on weather systems such as hurricanes (Larsen et al. 2007).
Chiroderma gorgasi was originally described by Handley (1960) using five specimens from the type locality in Panama. The author distinguished the new species from *C. trinitatum* by its smaller size, deeper brain case, shorter rostrum, shaper lacrimal ridge, bulging forehead, larger upper incisors, and thicker white band in the dorsal hairs. But at that time, *C. trinitatum* was only known by the holotype from Trinidad (Goodwin 1958) so the extent of variation within each species was poorly understood. Based on a specimen from Mitu in Amazonian Colombia, Barriga-Bonilla (1965) recognized the taxon as two subspecies and assigned his Colombian specimen to *C. t. gorgasi*. The subspecies were considered to be distributed from eastern Panama to western Venezuela for *C. t. gorgasi* and Trinidad to the Amazon basin for *C. t. trinitatum* (Jones and Carter 1976). However, with more geographic sampling the initial distinctions between the two taxa were less obvious due to individual and geographic variation (Simmons and Voss 1998), as also demonstrated by our morphometric analysis. But the taxonomy and distributional limits were still contentious with Gardner (2008) recognizing the Andes as the delineation of the subspecies and reassigning the specimen of Barriga-Bonilla (1965) from Mitu, Colombia, to *C. t. trinitatum*.

Our morphological review identified the presence of three cusps on the second lower premolar in cis-Andean populations referable to *C. trinitatum* and two cusps in trans-Andean populations referable to *C. gorgasi* that also match the taxonomic boundaries of Gardner (2008). Morphometrically, *C. trinitatum* averages smaller than *C. gorgasi* in all cranial measurements except for a proportionately broader braincase. Furthermore, our genetic analyses recovered *C. trinitatum* as the well-supported sister species to the larger and morphologically distinctive *C. doriae*, and not to the superficially similar *C. gorgasi*. Based on this morphological and molecular evidence, we recognize *C. gorgasi* as a distinct species and divergent lineage that does not share the most recent common ancestor with *C. trinitatum* (sensu stricto).

The overall topology of the Cytb tree proposed by Baker et al. (1994) is identical to our tree except for the recognition of *C. gorgasi*, which they did not have a sample of, as the sister species to *C. trinitatum* and *C. doriae*. The evolution of *Chiroderma* was suggested as occurring primarily by allopatric speciation (Baker et al. 1994). More specifically, *C. improvisum* arose by peripatric speciation in the Lesser Antilles after dispersing from its most recent common ancestor with *C. villosum* in South America. The Andes is an obvious geographic barrier separating *C. gorgasi* from the most recent common ancestor of *C. trinitatum* and *C. doriae*. A dated phylogeny is needed to test whether this is an older sundering event associated with the uplift of the northern Andes in the Late Miocene or a more recent dispersal event followed by isolation and the cessation of gene flow. Rojas et al. (2016) date the divergence of *Chiroderma* species to the Pliocene-Holocene, but *C. gorgasi* was not included in their dataset. The allopatric distribution of *C. trinitatum* and *C. doriae* suggests that perhaps the Cerrado Savanna in Brazil acted as a barrier after colonization of the Atlantic Forest from the Amazon, but the records of *C. doriae* for the Cerrado and the discovery of a species of *Chiroderma* in the dry deciduous forests of the Brazilian Caatinga, *C. vizottoi*, indicates that species of the genus can adapt to more harsh habitats. The speciation event that gave rise to *C. salvini* and the most recent common ancestor...
of the other species of *Chiroderma* is speculative without a thorough biogeographic analysis with a dated phylogeny.

Although not an overly species-rich genus, biodiversity surveys and molecular analyses are finding new distributional and taxonomic discoveries in *Chiroderma*. However, there are still large geographic gaps in sampling throughout the Neotropics, such as the Amazon basin in Brazil and northern South America in Colombia and Venezuela. In addition, this has hindered detailed study of the biogeography of the genus and more broadly the evolution of bats in the Neotropics.

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### Appendix 1

Tissue samples of *Chiroderma* used in the cytochrome c oxidase subunit 1 analysis.

| Sample ID | Species          | GenBank   | Country      | State/Department |
|-----------|------------------|-----------|--------------|------------------|
| ROM 111114 | *Chiroderma doriae* | JF448016  | Brazil       | Sao Paulo        |
| ROM 111141 | *Chiroderma doriae* | JF446371  | Brazil       | Sao Paulo        |
| ROM 111149 | *Chiroderma doriae* | JF446373  | Brazil       | Sao Paulo        |
| ROM 111163 | *Chiroderma doriae* | JF446372  | Brazil       | Sao Paulo        |
| ROM 105191 | *Chiroderma trinitatum* | JF448017  | Ecuador      | Napo             |
| ROM 105230 | *Chiroderma trinitatum* | JF448810  | Ecuador      | Napo             |
| ROM 105243 | *Chiroderma trinitatum* | JF448806  | Ecuador      | Napo             |
| ROM 105253 | *Chiroderma trinitatum* | JF448805  | Ecuador      | Napo             |
| ROM 105581 | *Chiroderma trinitatum* | JF448811  | Ecuador      | Napo             |
| ROM 105685 | *Chiroderma trinitatum* | JF448807  | Ecuador      | Napo             |
| ROM 105718 | *Chiroderma trinitatum* | JF448809  | Ecuador      | Napo             |
| ROM 105766 | *Chiroderma trinitatum* | JF448808  | Ecuador      | Napo             |
| ROM 106342 | *Chiroderma trinitatum* | JF448812  | Ecuador      | Napo             |
| ROM 104448 | *Chiroderma villosum* | JF448818  | Ecuador      | Napo             |
| ROM 104540 | *Chiroderma villosum* | JF448829  | Ecuador      | Napo             |
| ROM 104541 | *Chiroderma villosum* | JF448814  | Ecuador      | Napo             |
| ROM 104549 | *Chiroderma villosum* | JF448828  | Ecuador      | Napo             |
| ROM 105244 | *Chiroderma villosum* | JF448815  | Ecuador      | Napo             |
| ROM 105254 | *Chiroderma villosum* | JF448816  | Ecuador      | Napo             |
| ROM 105267 | *Chiroderma villosum* | JF448826  | Ecuador      | Napo             |
| ROM 105361 | *Chiroderma villosum* | JF448825  | Ecuador      | Napo             |
| ROM 105540 | *Chiroderma villosum* | JF448824  | Ecuador      | Napo             |
| ROM 105587 | *Chiroderma villosum* | JF448830  | Ecuador      | Napo             |
| ROM 105719 | *Chiroderma villosum* | JF448822  | Ecuador      | Napo             |
| ROM 105720 | *Chiroderma villosum* | JF448821  | Ecuador      | Napo             |
| ROM 105721 | *Chiroderma villosum* | JF448820  | Ecuador      | Napo             |
| ROM 105928 | *Chiroderma villosum* | JF448817  | Ecuador      | Napo             |
| ROM 105968 | *Chiroderma villosum* | JF448819  | Ecuador      | Napo             |
| ROM 1037400 | *Chiroderma villosum* | JF448827  | Ecuador      | Napo             |
| ROM 1037774 | *Chiroderma villosum* | JF448823  | Ecuador      | Napo             |
| ROM 101245 | *Chiroderma villosum* | JF446499  | El Salvador  | Ahuachapan       |
| ROM 99703  | *Chiroderma salvini* | JF446777  | Guatemala    | El Progreso      |
| ROM 103486 | *Chiroderma trinitatum* | JF454560  | Guyana       | Upper Demerara-Berbice |
| ROM 103503 | *Chiroderma trinitatum* | JF454561  | Guyana       | Upper Demerara-Berbice |
| ROM 103504 | *Chiroderma trinitatum* | MN714876  | Guyana       | Upper Demerara-Berbice |
| ROM 103505 | *Chiroderma trinitatum* | JF454562  | Guyana       | Upper Demerara-Berbice |
| ROM 107205 | *Chiroderma trinitatum* | EF080285  | Guyana       | Potaro-Siparuni  |
| ROM 107419 | *Chiroderma trinitatum* | EF080286  | Guyana       | Potaro-Siparuni  |
| ROM 107476 | *Chiroderma trinitatum* | MN714877  | Guyana       | Potaro-Siparuni  |
| ROM 108144 | *Chiroderma trinitatum* | JF454552  | Guyana       | Cuyuni-Mazaruni  |
| ROM 108244 | *Chiroderma trinitatum* | JF454559  | Guyana       | Cuyuni-Mazaruni  |
| Sample ID   | Species            | GenBank    | Country     | State/Department     |
|------------|--------------------|------------|-------------|----------------------|
| ROM 108463 | *Chiroderma trinitatum* | JF454544  | Guyana      | Potaro-Siparuni      |
| ROM 108554 | *Chiroderma trinitatum* | JF454545  | Guyana      | Potaro-Siparuni      |
| ROM 108587 | *Chiroderma trinitatum* | JF454555  | Guyana      | Potaro-Siparuni      |
| ROM 108588 | *Chiroderma trinitatum* | JF454554  | Guyana      | Potaro-Siparuni      |
| ROM 108714 | *Chiroderma trinitatum* | EF080287  | Guyana      | Potaro-Siparuni      |
| ROM 108763 | *Chiroderma trinitatum* | MN714878  | Guyana      | Potaro-Siparuni      |
| ROM 108889 | *Chiroderma trinitatum* | EF080288  | Guyana      | Potaro-Siparuni      |
| ROM 108950 | *Chiroderma trinitatum* | JF454557  | Guyana      | Potaro-Siparuni      |
| ROM 108993 | *Chiroderma trinitatum* | JF454556  | Guyana      | Potaro-Siparuni      |
| ROM 109026 | *Chiroderma trinitatum* | MN714879  | Guyana      | Potaro-Siparuni      |
| ROM 109195 | *Chiroderma trinitatum* | JF454558  | Guyana      | Potaro-Siparuni      |
| ROM 109271 | *Chiroderma trinitatum* | JF454553  | Guyana      | Potaro-Siparuni      |
| ROM 109333 | *Chiroderma trinitatum* | JF454542  | Guyana      | Potaro-Siparuni      |
| ROM 111627 | *Chiroderma trinitatum* | JF454543  | Guyana      | Potaro-Siparuni      |
| ROM 111809 | *Chiroderma trinitatum* | JF454547  | Guyana      | Potaro-Siparuni      |
| ROM 111844 | *Chiroderma trinitatum* | MN714880  | Guyana      | Potaro-Siparuni      |
| ROM 111884 | *Chiroderma trinitatum* | JF454546  | Guyana      | Potaro-Siparuni      |
| ROM 111946 | *Chiroderma trinitatum* | JF454548  | Guyana      | Potaro-Siparuni      |
| ROM 115807 | *Chiroderma trinitatum* | JF454550  | Guyana      | Potaro-Siparuni      |
| ROM 116630 | *Chiroderma trinitatum* | JF454549  | Guyana      | Potaro-Siparuni      |
| ROM 118996 | *Chiroderma trinitatum* | JF454551  | Guyana      | Upper Takutu-Upper Essequibo |
| ROM 121975 | *Chiroderma trinitatum* | MN714881  | Guyana      | Potaro-Siparuni      |
| ROM 125124 | *Chiroderma trinitatum* | MN714882  | Guyana      | Potaro-Siparuni      |
| ROM 103214 | *Chiroderma villosum*   | JF454584  | Guyana      | Upper Takutu-Upper Essequibo |
| ROM 103331 | *Chiroderma villosum*   | JF454585  | Guyana      | Upper Takutu-Upper Essequibo |
| ROM 106644 | *Chiroderma villosum*   | JF454566  | Guyana      | Upper Takutu-Upper Essequibo |
| ROM 107111 | *Chiroderma villosum*   | EF080290  | Guyana      | Potaro-Siparuni      |
| ROM 107112 | *Chiroderma villosum*   | EF080291  | Guyana      | Potaro-Siparuni      |
| ROM 107394 | *Chiroderma villosum*   | EF080292  | Guyana      | Potaro-Siparuni      |
| ROM 108203 | *Chiroderma villosum*   | JF454565  | Guyana      | Cuyuni-Mazaruni      |
| ROM 108219 | *Chiroderma villosum*   | JF454564  | Guyana      | Cuyuni-Mazaruni      |
| ROM 108764 | *Chiroderma villosum*   | JF454571  | Guyana      | Potaro-Siparuni      |
| ROM 108765 | *Chiroderma villosum*   | JF454570  | Guyana      | Potaro-Siparuni      |
| ROM 108843 | *Chiroderma villosum*   | EF080289  | Guyana      | Potaro-Siparuni      |
| ROM 108998 | *Chiroderma villosum*   | JF454573  | Guyana      | Potaro-Siparuni      |
| ROM 109138 | *Chiroderma villosum*   | JF454572  | Guyana      | Potaro-Siparuni      |
| ROM 109175 | *Chiroderma villosum*   | JF454569  | Guyana      | Potaro-Siparuni      |
| ROM 109221 | *Chiroderma villosum*   | JF454568  | Guyana      | Potaro-Siparuni      |
| ROM 109270 | *Chiroderma villosum*   | JF454567  | Guyana      | Potaro-Siparuni      |
| ROM 109307 | *Chiroderma villosum*   | JF454583  | Guyana      | Potaro-Siparuni      |
| ROM 109308 | *Chiroderma villosum*   | JF454582  | Guyana      | Potaro-Siparuni      |
| ROM 109337 | *Chiroderma villosum*   | JF454581  | Guyana      | Potaro-Siparuni      |
| ROM 111628 | *Chiroderma villosum*   | EF080293  | Guyana      | Potaro-Siparuni      |
| ROM 111629 | *Chiroderma villosum*   | JF459119  | Guyana      | Potaro-Siparuni      |
| ROM 111754 | *Chiroderma villosum*   | JF454580  | Guyana      | Potaro-Siparuni      |
| ROM 111768 | *Chiroderma villosum*   | JF454579  | Guyana      | Potaro-Siparuni      |
| ROM 111769 | *Chiroderma villosum*   | JF454578  | Guyana      | Potaro-Siparuni      |
| ROM 111770 | *Chiroderma villosum*   | JF454577  | Guyana      | Potaro-Siparuni      |
| ROM 111788 | *Chiroderma villosum*   | JF454576  | Guyana      | Potaro-Siparuni      |
| ROM 111836 | *Chiroderma villosum*   | JF454575  | Guyana      | Potaro-Siparuni      |
| Sample ID  | Species                  | GenBank  | Country          | State/Department          |
|------------|--------------------------|----------|------------------|---------------------------|
| ROM 111845 | Chiroderma villosum      | JF454574 | Guyana           | Potaro-Siparuni           |
| ROM 119167 | Chiroderma villosum      | MN714883 | Guyana           | Upper Takutu-Upper Essequibo |
| ROM 119230 | Chiroderma villosum      | JF454586 | Guyana           | Upper Takutu-Upper Essequibo |
| ROM 122481 | Chiroderma villosum      | MN714884 | Guyana           | Potaro-Siparuni           |
| ROM 98850  | Chiroderma villosum      | JF454563 | Guyana           | Barima-Waini              |
| ROM 125179 | Chiroderma villosum      | MN714885 | Guyana           | East Berbice-Corentyne    |
| ROM F38952 | Chiroderma villosum      | MN714886 | Guyana           | Potaro-Siparuni           |
| ROM 98702  | Uroderma bilobatum       | JF435925 | Guyana           | Barima-Waini              |
| ROM 96536  | Chiroderma villosum      | JF448018 | Mexico           | Campeche                  |
| ROM FN30654| Chiroderma villosum      | JF447242 | Mexico           | Campeche                  |
| ROM 104342 | Chiroderma gorgasi       | MN714901 | Panama           | Darien                    |
| ROM 104352 | Chiroderma villosum      | JF447405 | Panama           | Darien                    |
| ROM 122084 | Chiroderma trinitatum    | MN714887 | Peru             | Loreto                    |
| ROM 122137 | Chiroderma trinitatum    | MN714888 | Peru             | Loreto                    |
| ROM 122149 | Chiroderma trinitatum    | MN714889 | Peru             | Loreto                    |
| ROM 122165 | Chiroderma villosum      | MN714890 | Peru             | Loreto                    |
| ROM 122260 | Chiroderma villosum      | MN714891 | Peru             | Loreto                    |
| ROM 125567 | Chiroderma villosum      | MN714892 | Peru             | Tumbes                    |
| ROM 126002 | Chiroderma improvisum    | MN714893 | Nevis            | Saint Thomas Lowland Parish |
| ROM 114170 | Chiroderma trinitatum    | JF447622 | Suriname         | Brokopondo                |
| ROM 114213 | Chiroderma trinitatum    | JF447625 | Suriname         | Brokopondo                |
| ROM 114233 | Chiroderma trinitatum    | JF447623 | Suriname         | Brokopondo                |
| ROM 114234 | Chiroderma trinitatum    | JF447624 | Suriname         | Brokopondo                |
| ROM 117003 | Chiroderma trinitatum    | JF447627 | Suriname         | Sipaliwini                |
| ROM 117003 | Chiroderma trinitatum    | MN714894 | Suriname         | Sipaliwini                |
| ROM 117027 | Chiroderma trinitatum    | JF447626 | Suriname         | Sipaliwini                |
| ROM 117083 | Chiroderma trinitatum    | JF447628 | Suriname         | Sipaliwini                |
| ROM 117376 | Chiroderma trinitatum    | EU096695 | Suriname         | Sipaliwini                |
| ROM 117555 | Chiroderma trinitatum    | EU096696 | Suriname         | Sipaliwini                |
| ROM 120098 | Chiroderma trinitatum    | MN714895 | Suriname         | Sipaliwini                |
| ROM 120168 | Chiroderma trinitatum    | HQ545629 | Suriname         | Sipaliwini                |
| ROM 120225 | Chiroderma trinitatum    | HQ545678 | Suriname         | Sipaliwini                |
| ROM 120384 | Chiroderma trinitatum    | HQ919736 | Suriname         | Sipaliwini                |
| ROM 114212 | Chiroderma villosum      | JF447630 | Suriname         | Brokopondo                |
| ROM 114228 | Chiroderma villosum      | JF447631 | Suriname         | Brokopondo                |
| ROM 117119 | Chiroderma villosum      | JF447629 | Suriname         | Sipaliwini                |
| ROM 117375 | Chiroderma villosum      | EU096697 | Suriname         | Sipaliwini                |
| ROM 120226 | Chiroderma villosum      | HQ545679 | Suriname         | Sipaliwini                |
| ROM 120239 | Chiroderma villosum      | HQ545445 | Suriname         | Sipaliwini                |
| ROM 120240 | Chiroderma villosum      | HQ545446 | Suriname         | Sipaliwini                |
| ROM 120354 | Chiroderma villosum      | MN714896 | Suriname         | Sipaliwini                |
| ROM 120364 | Chiroderma villosum      | HQ919717 | Suriname         | Sipaliwini                |
| ROM 121027 | Chiroderma villosum      | MN714897 | Suriname         | Sipaliwini                |
| ROM 121117 | Chiroderma villosum      | MN714898 | Suriname         | Sipaliwini                |
| ROM 126174 | Chiroderma villosum      | MN714899 | Suriname         | Para                      |
| ROM 113919 | Platyrhinus incarum      | JF435616 | Suriname         | Brokopondo                |
| ACUNHC 393 | Chiroderma villosum      | MN714900 | Venezuela        | Amazonas                  |
Cryptic diversity and range extension in the big-eyed bat genus Chiroderma

Appendix 2

Tissue samples of Chiroderma used in the cytochrome b analysis.

| Sample ID  | Species                  | GenBank  | Country  | State/Province |
|------------|--------------------------|----------|----------|----------------|
| UNESP 16506| Chiroderma doriae        | L28937   | Brazil   | Sao Paulo      |
| TK 16379   | Chiroderma doriae        | AY169958 | Brazil   |                |
| TK 15713   | Chiroderma improvisum    | L28938   | Montserrat| St. Anthony   |
| TK 25052   | Chiroderma villoum       | DQ312414 | Trinidad | George         |
| FMNH 174652| Chiroderma villoum       | FJ154121 | Peru     | Madre de Dios  |
| TK 17627   | Platyrrhinus belleri     | L28940   | Suriname | Marowijne      |
| TK 25256   | Uroderma bilobatum       | L28941   | Trinidad | St. George     |
| TK 22581   | Chiroderma salvini       | L28939   | Panama   | Darien         |
| TK 25211   | Chiroderma trinitatum    | DQ312413 | Trinidad | St. George     |
| ASK 7799   | Chiroderma villoum       | JF442196 | Ecuador  | Orellana       |
| ASK 7667   | Chiroderma villoum       | JF442139 | Ecuador  | Napo           |
| MN 36375   | Chiroderma villoum       | DQ903823 | Brazil   |               |
| SK-Bat-61  | Chiroderma improvisum    | JQ915203 |         | Saint Kitts    |
| ROM 104342 | Chiroderma gorgasi       | MN714902 | Panama   | Darien         |

Appendix 3

Specimens of Chiroderma examined morphologically. Vouchers examined are arranged alphabetically by species and country. See “Material and Methods” for collection acronyms.

Chiroderma doriae – Brazil: São Paulo - ROM 111163, ROM 111141, ROM 111114, ROM 111149.

Chiroderma improvisum – Montserrat: St. Anthony Parish – TTU 31403; St. Kitts and Nevis: Barnes Ghaut - ROM 126002.

Chiroderma salvini – El Salvador: Morazan - ROM 83365, ROM 85948, Santa Ana - ROM 101526; Guatemala: El Progreso - ROM 99703; Panama: Darien - ROM 78472, ROM 91194.

Chiroderma trinitatum gorgasi – Colombia: Valle del Cauca - USNM 483763, USNM 483765, Antioquia - USNM 499478, USNM 499476; Panama: Bocas Del Toro – USNM 319498, USNM 335295, Darien - FMNH 128132, ROM 104342, USNM 309901, USNM 309903-holotype, San Blas - USNM 309905.

Chiroderma trinitatum trinitatum – Colombia: Vaupes - ROM 45276, ROM 45278, ROM 45280, ROM 45281, ROM 45284, Putumayo – ROM 63236, ROM 63237, ROM 63238; Ecuador: Napo - ROM 105191, ROM 105243, ROM 105253, ROM 105685, ROM 105766, ROM 106342; Guyana: Cuyuni-Mazaruni - ROM 108144, Demerara-Berbice - ROM 57392, ROM 103486, ROM 103503, Upper, Potaro-Siparuni - ROM 107205, ROM 107419, ROM 107476, ROM 108463, ROM 108554, ROM 108587, ROM 108714, ROM 108763, ROM 108889,
ROM 108950, ROM 108993, ROM 109195, ROM 109333, ROM 111627, ROM 111809, ROM 111844, ROM 111946, ROM 115807, ROM 116630; Suriname: Brokopondo - ROM 114170, ROM 114213, ROM 114233, ROM 114234, Sipaliwini - ROM 117027, ROM 117376, ROM 120168, ROM 120225, ROM 120384; Trinidad: Saint Andrew County – AMNH 175325-holotype.

Chiroderma villosum – Bolivia: Carrasco - ROM 78471; Colombia: Choco - ROM 85849, Vaupes - ROM 44952, ROM 44953, ROM 44954, ROM 45243, ROM 45245, ROM 45246, ROM 45247, ROM 45249, ROM 45250, ROM 45251, ROM 45252, ROM 45253, ROM 45254, ROM 45255, ROM 45257; Ecuador: Napo - ROM 104448, ROM 104541, ROM 104549, ROM 105244, ROM 105254, ROM 105361, ROM 105720, ROM 105721); Guyana: Barima-Waini - ROM 98850, Potaro-Siparuni - ROM 107111, ROM 107112, ROM 107394, ROM 108219, ROM 108764, ROM 108843, ROM 108998, ROM 109138, ROM 109175, ROM 109221, ROM 109307, ROM 109308, ROM 109337, ROM 111628, ROM 111629, ROM 111754, ROM 111768, ROM 111769, ROM 111770, ROM 111788, ROM 111836, ROM 111845, ROM 122481, Upper Demerara-Berbice - ROM 60402, ROM 60423, Upper Takutu-Upper Essequibo - ROM 35614, ROM 103214, ROM 106644, ROM 119167, ROM 119230; Panama: Darien - ROM 104352; Suriname: Brokopondo - ROM 114212, Sipaliwini - ROM 117119, ROM 117375, ROM 120226, ROM 120239, ROM 120364, ROM 121027; Trinidad and Tobago: Nariva - ROM 124684, ROM 124691.

Supplementary material I

Maximum likelihood tree of cytochrome c oxidase subunit 1 gene for big-eyed bats Chiroderma as presented in Fig. 3, but expanded to show individuals
Authors: Burton K. Lim, Livia O. Loureiro, Guilherme S. T. Garbino
Data type: phylogenetic dendrogram
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Link: https://doi.org/10.3897/zookeys.918.48786.suppl1
Supplementary material 2

**Maximum parsimony tree of cytochrome c oxidase subunit 1 gene for *Chiroderma*. Bootstrap percentages show support at each node**

Authors: Burton K. Lim, Livia O. Loureiro, Guilherme S. T. Garbino
Data type: phylogenetic dendrogram
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Supplementary material 3

**Maximum likelihood tree of cytochrome b gene for *Chiroderma*. Bootstrap percentages show support at each node**

Authors: Burton K. Lim, Livia O. Loureiro, Guilherme S. T. Garbino
Data type: phylogenetic dendrogram
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Supplementary material 4

**Maximum parsimony tree of cytochrome b gene for *Chiroderma*. Bootstrap percentages show support at each node**

Authors: Burton K. Lim, Livia O. Loureiro, Guilherme S. T. Garbino
Data type: phylogenetic dendrogram
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