The taxonomic status of *Myotis nesopolus larensis* (Chiroptera, Vespertilionidae) and new insights on the diversity of Caribbean *Myotis*

Roberto Leonan M. Novaes¹, Vinícius C. Cláudio¹², Roxanne J. Larsen³, Don E. Wilson², Marcelo Weksler⁴, Ricardo Moratelli⁵

1 Universidade Federal do Rio de Janeiro, Programa de Pós-Graduação em Biodiversidade e Biologia Evolutiva. Av. Carlos Chagas Filho 373, Cidade Universitária, 21941-902, Rio de Janeiro, RJ, Brazil 2 Smithsonian Institution, National Museum of Natural History, Division of Mammals. 10th St. & Constitution Ave. NW, 20013-7012, Washington, DC, USA 3 University of Minnesota, College of Veterinary Medicine, 1365 Gortner Ave., 55108, Saint Paul, MN, USA 4 Museu Nacional / Universidade Federal do Rio de Janeiro, Departamento de Vertebrados. Quinta da Boa Vista s/n, São Cristóvão, 20940-040, Rio de Janeiro, RJ, Brazil 5 Fundação Oswaldo Cruz, Fiocruz Mata Atlântica. R. Sampaio Correa s/n, Taquara, 22713-560, Rio de Janeiro, RJ, Brazil

Corresponding author: Roberto Leonan M. Novaes (robertoleonan@gmail.com)

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**Abstract**

*Myotis nesopolus* currently comprises two subspecies. The nominate subspecies (*M. n. nesopolus*) occurs on the Caribbean islands of Curaçao and Bonaire, Netherlands Antilles, whereas *M. n. larensis* is known from mainland South America in northeastern Colombia and northwestern Venezuela. Our Maximum Likelihood phylogenetic analyses of cytochrome-b gene sequences recovered *M. nesopolus* as a paraphyletic group, with *M. n. nesopolus* and *M. n. larensis* as non-sister lineages. The haplotype network indicates that these two subspecies do not share any haplotypes and are in different evolutionary trajectories. Additionally, these two subspecies can be distinguished on the basis of qualitative and quantitative morphological traits. This pattern supports the recognition of *M. nesopolus* and *M. larensis* as full species. Our results also reveal that the assemblage of Caribbean *Myotis* do not form a monophyletic group. Caribbean species are phylogenetically close to mainland species from northern South America and Central America, suggesting that colonization of Caribbean islands happened multiple times.
Resumo
Atualmente *Myotis nesopolus* compreende duas subespécies: *M. n. nesopolus* ocorre nas ilhas caribenhas de Curaçao e Bonaire, Antilhas Holandesas, enquanto *M. n. larensis* é conhecido para o continente da América do Sul, no nordeste da Colômbia e noroeste da Venezuela. Nossa inferência filogenética por Máxima Verossimilhança recuperou *M. nesopolus* como parafilética, com *M. n. nesopolus* e *M. n. larensis* sendo linhagens não-irmãs. Além disso, essas duas subespécies não compartilham nenhum haplótipo. Adicionalmente, as subespécies podem ser diferenciadas a partir de caracteres morfológicos e morfométricos. Esse achado suporta o reconhecimento de *M. nesopolus* e *M. larensis* como espécies distintas. Nossos resultados revelam que os *Myotis* do Caribe não formam um grupo monofilético. Espécies caribenhas são filogeneticamente próximas de espécies continentais das Américas Central e do Sul, sugerindo que a colonização das ilhas do Caribe aconteceu por múltiplos eventos de dispersão.

Keywords
Bats, biogeography, Lesser Antilles, morphology, morphometry, taxonomy, South America, Venezuela

Introduction

*Myotis* Kaup, 1829 (Vespertilionidae, Myotinae) comprises more than 120 species distributed worldwide, and is the most speciose genus of bats (Simmons 2005; Burgin et al. 2018). Twenty-seven species are recognized from the Neotropics (Wilson 2008; Moratelli et al. 2017, 2019a; Carrión-Bonilla and Cook 2020). However, molecular evidence has revealed that the current species richness is underestimated (Claire et al. 2011; Larsen et al. 2012a; Chaverri et al. 2016; Moratelli et al. 2017).

Two subspecies of *Myotis nesopolus* Miller, 1900 are recognized. The nominate subspecies, *M. n. nesopolus*, is known from Curaçao and Bonaire in the Netherlands Antilles. The other subspecies, *M. n. larensis* LaVal, 1973, is known from mainland South America in northeastern Colombia and northwestern Venezuela (LaVal 1973; Wilson 2008; Muñoz-Garay and Mantilla-Meluk 2012; Moratelli et al. 2013). LaVal (1973) described *Myotis larensis* as a full species from “Río Tocuyo, Lara, Venezuela”. Genoways and Williams (1979), however, treat *larensis* as a subspecies of *Myotis nesopolus*. Miller’s (1900) description of *M. nesopolus* was based on one specimen from Willemstad, Curaçao, Netherlands Antilles. Subsequently, Genoways and Williams (1979) considered that representatives of *Myotis* from Bonaire island, originally identified as *Myotis nigricans* (Schinz, 1821), were misidentifications of *M. nesopolus*, which was confirmed by Moratelli et al. (2017).

Previous molecular and morphological studies questioned the subspecific status of mainland populations of *M. nesopolus*, suggesting that the two subspecies might represent different species (Larsen et al. 2012b; Moratelli et al. 2013, 2017). Here we reassess the taxonomic status of *M. n. larensis* in the light of new morphological and genetic analyses.
Materials and methods

Specimens examined

Specimens of *M. nesopolus* used in this study are deposited in the American Museum of Natural History (*AMNH*, New York, USA), Carnegie Museum of Natural History (*CM*, Pittsburgh, USA), Smithsonian’s National Museum of Natural History (*USNM*, Washington DC, USA), and Museum of Texas Tech University (*TTU*, Lubbock, USA). We examined the holotype of *M. n. nesopolus* (*USNM* 101849), two topotypes from Curaçao (*CM* 52432, *USNM* 105128), and nine specimens from Bonaire (Appendix 1). Material of *M. n. larensis* includes the holotype (*AMNH* 130709), and fifteen additional specimens from mainland Venezuela.

Molecular analyses

Phylogenetic analyses of complete cytochrome-b gene (cyt-b, 1,140 bp, no gaps) sequences were conducted for the Neotropical assemblage of *Myotis*. A total of 122 sequences, including outgroups, were retrieved from GenBank (Appendix 2). We used the palearctic species *Myotis brandtii* (Eversmann, 1845) and *Myotis gracilis* Ognev, 1927 as outgroups because they are sister to the Neotropical clade (see Ruedi et al. 2013). Multiple sequence alignment of full length cyt-b sequences were performed with MEGA X (Kumar et al. 2018), using MUSCLE algorithm with default settings (Edgar 2004). Subsequently, the Bayesian Information Criterion (BIC), as implemented in JModelTest2 (Darriba et al. 2012), was used to determine the best-fit models of nucleotide substitution. The Hasegawa-Kishino-Yano model (Hasegawa et al. 1985) was chosen to correct the heterogeneity rate using gamma-distribution with invariant sites (i.e., HKY + Γ + I).

The phylogenetic analysis was carried out using Maximum Likelihood (ML) method (Felsenstein 1981), in the software RAxML v8.0 (Stamatakis 2014). To assess the nodal support, we calculated a nonparametric bootstrap using 1000 replications. Genetic distance values for cyt-b sequences were calculated in MEGA X using the Kimura 2-parameter model (Kimura 1980).

To understand the population structure of *M. n. nesopolus*, *M. n. larensis* and other phylogenetically related population groups, we built a haplotype network (distribution of haplotypes by previously defined population groups) using the median-joining algorithm in the Network 4.6.1.3 software (Bandelt et al. 1999).

Morphological and morphometric analyses

We examined 284 specimens for the morphological comparisons, including *M. n. nesopolus* (*N* = 10), *M. n. larensis* (*N* = 9) and 14 species of Neotropical *Myotis* deposited in 11 collections in Brazil, Canada and United States (Appendix 1). Specimens were
identified following Wilson (2008) and Moratelli et al. (2011, 2013, 2017). The main qualitative morphological characters used in the comparisons were: (i) presence and height of sagittal crest; (ii) presence and height of lambdoidal crests; (iii) inclination shape of the frontal and parietal bones; (iv) presence of a fringe of hairs along the trailing edge of the uropatagium; (v) dorsal and ventral fur texture and height; (vi) pattern of fur coloring, with the capitalized color nomenclature following Ridgway (1912).

We took one external and 16 craniodental measurements (Table 1), using digital calipers to the nearest 0.01 mm. Measurements were made under binocular microscopes with low magnification (usually 6×). Measurements were recorded from adults and are reported in millimeters (mm). The length of ear and body mass were recorded from skin labels. We used a principal component analysis (PCA) to identify general trends of cranial size and shape variation among samples, and a discriminant function analysis (DFA), with a priori identification of samples, to compare skull size and shape of *M. n. nesopolus* (*N* = 9) and *M. n. larensis* (*N* = 9). For these analyses, we selected a subset of 11 craniodental dimensions representing different axes of the length and width of skull, rostrum, and mandible, as follows: greatest length of skull, including incisors (GLS), condylo-incisive length (CIL), mastoid breadth (MAB), braincase breadth (BCB), interorbital breadth (IOB), postorbital breadth (POB), breadth across canines (BAC), breadth across molars (BAM), maxillary toothrow length (MTL), molariform toothrow length (M1–M3), and mandibular toothrow length (MAN). PCA and DFA analyses were run in R software (R Development Core Team 2012) using the MASS and Lattice packages (Venables and Ripley 2002; Sarkar 2008). Because multivariate procedures require complete data sets, missing values (ca 1.5% of the total dataset) were estimated from the existing raw data using the Amelia II package (Honaker

### Table 1. Description of cranial, mandibular, and external dimensions (and their abbreviations). Lengths were measured from the anteriormost point or surface of the 1<sup>st</sup> structure to the posteriormost point or surface of the 2<sup>nd</sup> structure, except as specified.

| Measurements               | Acronyms | Descriptions                                                                 |
|----------------------------|----------|-------------------------------------------------------------------------------|
| Forearm length             | FA       | From the elbow to the distal end of the forearm including carpals             |
| Greatest length of skull   | GLS      | From the apex of the upper internal incisors, to the occiput                  |
| Condylo-canine length      | CCL      | From the anterior surface of the upper canines to a line connecting the occipital condyles |
| Condylo-basal length       | CBL      | From the premaxillae to a line connecting the occipital condyles               |
| Condylo-incisive length    | CIL      | From the apex of upper internal incisors to a line connecting the occipital condyles |
| Basal length               | BAL      | Least distance from the apex of upper internal incisors to the ventral margin of the foramen magnum |
| Zygomatic breadth          | ZYG      | Greatest breadth across the outer margins of the zygomatic arches             |
| Mastoid breadth            | MAB      | Greatest breadth across the mastoid region                                     |
| Braincase breadth          | BCB      | Greatest breadth of the globular part of the braincase                        |
| Interorbital breadth       | IOB      | Least breadth between the orbits                                              |
| Postorbital breadth        | POB      | Least breadth across frontals posterior to the postorbital bulges             |
| Breadth across canines     | BAC      | Greatest breadth across outer edges of the crowns of upper canines, including cingulae |
| Breadth across molars      | BAM      | Greatest breadth across outer edges of the crowns of upper molars             |
| Maxillary toothrow length  | MTL      | From the upper canine to M3                                                   |
| Molariform toothrow length | M1–M3    | From M1 to M3                                                                  |
| Mandibular length          | MAL      | From the mandibular symphysis to the condyloid process                        |
| Mandibular toothrow length | MAN      | From the lower canine to m3                                                   |
et al. 2011) implemented in R software. Measurements were transformed to natural
logs and covariance matrices were computed considering all variables. Subsequently,
an analysis of variance using Mann-Whitney statistics was employed to test whether
the population samples differ in cranial dimensions. The comparison was made using
p-values and when less than 0.001 were considered as statistically significant. This
analysis was run in the software PAST 3.3 (Hammer et al. 2001).

Results

Molecular analyses

The ML phylogeny based on cyt-b sequences indicates that *M. nesopolus*, as currently
recognized, is paraphyletic, with *M. n. nesopolus* more closely related to an eastern Pe-
ruvian unidentified lineage, whereas *M. n. larensis* was recovered more closely related
to an unidentified lineage from western Ecuador (Fig. 1), although this phylogeny and
branching events has low nodal support. These unidentified species from Peru and
Ecuador were originally designated as *Myotis nigricans* by the original collector due to
morphological similarities. However, *M. nigricans* has been recovered as polyphyletic
and considered a cryptic species complex in many studies (Moratelli et al. 2011, 2013,
2016, 2017; Larsen et al. 2012a). Therefore, we decided not to give a name to the line-
ages related to *M. nesopolus* and *M. larensis*. We emphasize that the previous identifica-
tion of these specimens as *M. nigricans* by one of our authors (RJL) in a previous study
(Larsen et al. 2012a) indicates that these populations are morphologically distinct from
those considered here as *M. nesopolus* and *M. larensis*.

The Caribbean *Myotis* species do not form a monophyletic group, being related
to *Myotis atacamensis* (Lataste, 1892) and other mainland putative species. Neverthe-
less, the phylogenetic relationship of Caribbean *Myotis* clade is not fully resolved,
since a polytomy was recovered among *M*. sp. 3 from Honduras and the ancestral
lineage of *M. n. nesopolus* and *M*. sp. 2 from Peru, and of *M. n. larensis* and *M*. sp. 1
from Ecuador. Similarly, a polytomy was recovered among *M. atacamensis*, *M. mar-
tiniquensis* and an ancestral lineage of *M. dominicensis*, *M. nyctor* and *M*. sp. 4 from
Suriname (Fig. 1).

The average cyt-b pairwise distance between *M. n. larensis* and *Myotis* sp. 1 from
western Ecuador is 2.1% ± 0.3; between *M. n. nesopolus* and *Myotis* sp. 2 from eastern
Peru is 3.8% ± 0.4; and between *M. n. nesopolus* and *M. n. larensis* is 4.0% ± 0.3
(Table 2). Levels of intraspecific variation were less than 0.8% for all recognized and
putative species (Table 2).

The haplotype network indicates that there are no haplotypes shared between
*M. n. nesopolus*, *M. n. larensis*, and phylogenetically close species (Fig. 2). The haplo-
types were grouped into small clusters well-distributed among the populations, with no
central haplotype. The network indicates spatial structuring with isolation among the
population groups tested, agreeing with what was obtained by phylogenetic inference.
Figure 1. Phylogenetic tree resulting from the Maximum Likelihood analysis of cytochrome-b sequences of species of *Myotis*. Nodal support was calculated by bootstrap and black solid circles are values between 100–95% and hollow white circle are values between 94–90%. Values less than 90% were not indicated. The rectangle encloses the phylogenetic relationship, where branches were transformed to cladogram, among *M. nesopolus*, *M. larenis*, Caribbean *Myotis* (colored terminals) and mainland haplogroups of five more closely related species and candidate species.

**Morphological analyses**

The first principal component (PC1) accounted for 87% of the total craniometric variation, and represents overall skull size (Fig. 3A, B). Along this axis, scores of *M. n. larenis* and *M. n. nesopolus* do not overlap. On the other hand, the two samples overlap broadly along the second principal component (PC2 = 5%) which represents overall skull shape. The distribution of *M. n. larenis* and *M. n. nesopolus* samples across size and shape axes in the discriminant analysis (Fig. 3C, D) is similar to that observed in
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**Table 2.** Average Kimura 2-parameter genetic distances within (along diagonal) and among (below diagonal) *Myotis* taxa based on cytochrome-b gene sequences. Boldface value indicates the distance between *M. larensis* and *M. nesopolus*. Hyphen indicates groups with a single sequence.

| Taxa                          | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   |
|-------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|
| *M. atacamensis* (Peru)       | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    | –    |
| *Myotis* sp. 4 (Suriname)     | 0.085| 0.002|      |      |      |      |      |      |      |      |      |      |
| *M. nector* (Grenada)         | 0.103| 0.080|      |      |      |      |      |      |      |      |      |      |
| *M. nector* (Barbados)        | 0.089| 0.070| 0.002| 0.004|      |      |      |      |      |      |      |      |
| *M. dominicensis* (Dominica)  | 0.080| 0.087| 0.092| 0.088| 0.001|      |      |      |      |      |      |      |
| *M. martiniquensis* (Martinique) | 0.087| 0.093| 0.089| 0.094| 0.887| 0.002|      |      |      |      |      |      |
| *M. n. larensis* (Venezuela)  | 0.093| 0.107| 0.127| 0.119| 0.097| 0.096| 0.003|      |      |      |      |      |
| *Myotis* sp. 1 (W Ecuador)    | 0.091| 0.104| 0.134| 0.120| 0.092| 0.093| 0.021| 0.002|      |      |      |      |
| *Myotis* sp. 2 (E Peru)       | 0.104| 0.115| 0.138| 0.126| 0.107| 0.104| 0.034| 0.033| 0.001|      |      |      |
| *M. n. nesopolus* (Bonaire)   | 0.103| 0.115| 0.147| 0.124| 0.104| 0.106| 0.040| 0.044| 0.038| 0.008|      |      |
| *Myotis* sp. 3 (Honduras)     | 0.103| 0.116| 0.133| 0.120| 0.107| 0.105| 0.046| 0.049| 0.056| 0.053|      |      |
| *M. attenboroughi* (Tobago)   | 0.081| 0.093| 0.101| 0.099| 0.091| 0.088| 0.068| 0.075| 0.076| 0.078| 0.079| 0.000|

*Figure 2.* Haplotype network from cyt-b sequences of *Myotis nesopolus* (blue), *Myotis larensis* (red) and other mainland closest *Myotis* lineages from Central and South America. Each tick mark represents a single base-pair mutation.
Figure 3. Plots showing convex-hulls and vector correlation of cranial measurements of Principal Component Analysis (A, B) and Discriminant Function Analysis (C, D) for *Myotis nesopolus* from Curaçao (black square), *Myotis nesopolus* from Bonaire (blue triangles) and *Myotis larensis* from Venezuela mainland (red dots).

The PCA. Measurements associated with skull and mandible length (GLS, CIL, MAN) and skull width (IOB) were the most useful to discriminate samples (Table 3). Considering that skull axes are represented by the set of measurements used in the morphometric multivariate analysis, these results reveal that *M. n. larensis* and *M. n. nesopolus* have distinct skull size and shape.

Populations from the Antilles and mainland South America do not overlap in measurements of several characters, which may be useful in distinguishing species: *M. n. larensis* forearm length ranges from 31.2 to 33.2 mm, and GLS from 13.6 to 14.5 mm; *M. n. nesopolus* forearm length ranges from 28.2 to 31.0 mm, and GLS from 12.9 to 13.4 mm. The Mann-Whitney test found significant differences in 11 of the 14 measurements tested (Table 4).

Population samples from the Antilles and mainland South America have several qualitative morphological differences. Specimens of *M. n. nesopolus* have moderately
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**Table 3.** Vector correlation loadings with original variables of principal components (PC1 and PC2) and discriminant functions (DF1 and DF2) for selected samples of *M. larensis* and *M. nesopolus*. See Table 1 for variable abbreviations.

| Measurements | PC1   | PC2  | DF1   | DF2   |
|--------------|-------|------|-------|-------|
| MAN          | 0.324 | -0.091 | 0.063 | 0.016 |
| GLS          | 0.573 | -0.103 | 0.109 | 0.026 |
| CIL          | 0.506 | -0.056 | 0.093 | 0.027 |
| MAB          | 0.097 | 0.327 | 0.012 | 0.012 |
| BCB          | 0.109 | 0.108 | 0.019 | 0.003 |
| IOB          | 0.258 | 0.775 | 0.051 | 0.014 |
| POB          | -0.02 | 0.363 | -0.005 | 0.026 |
| BAC          | 0.198 | 0.031 | 0.04  | 0.021 |
| BAM          | 0.277 | -0.165 | 0.059 | -0.015 |
| MTL          | 0.262 | -0.088 | 0.052 | 0.011 |
| M1–3         | 0.187 | -0.298 | 0.040 | -0.007 |

**Table 4.** Selected measurements (mm) of *M. larensis* from Venezuela and *M. nesopolus* from Curaçao and Bonaire. Descriptive statistics include the mean, range (in parentheses), and sample size. See Table 1 for variable abbreviations. Mann-Whitney Test *p*-values was used to compare cranial measurements between samples. Measurements with hyphen (–) not were tested due to disparate samples size.

| Measurements | *Myotis larensis* | *Myotis nesopolus* | *P*-value |
|--------------|-------------------|-------------------|-----------|
| FA           | 32.2 (31.2–33.2) 7 | 29.7 (28.2–31.0) 11 | –         |
| GLS          | 13.7 (13.3–14.4) 9 | 12.9 (12.8–13.1) 9 | < 0.001   |
| CCL          | 12.1 (11.5–12.7) 9 | 11.6 (11.4–11.8) 9 | < 0.001   |
| CRL          | 12.8 (12.4–13.5) 9 | 12.2 (12.0–12.5) 9 | < 0.001   |
| CIL          | 12.9 (12.6–13.6) 9 | 12.4 (12.2–12.6) 9 | < 0.001   |
| BAL          | 11.6 (11.2–12.4) 9 | 11.1 (10.9–11.3) 9 | < 0.001   |
| ZYG          | 8.1 (8.0–8.2) 3    | 7.8 (7.7–8.0) 8    | –         |
| MAB          | 5.3 (5.1–5.6) 9    | 6.7 (6.4–6.8) 9    | 0.247     |
| BCB          | 6.2 (6.1–6.3) 9    | 6.1 (5.9–6.2) 9    | 0.017     |
| IOB          | 4.4 (4.0–4.7) 9    | 4.0 (3.9–4.2) 9    | 0.003     |
| POB          | 3.3 (3.2–3.4) 9    | 3.3 (3.2–3.5) 9    | 0.374     |
| BAC          | 3.3 (3.2–3.5) 9    | 3.0 (3.0–3.2) 9    | < 0.001   |
| BAM          | 5.3 (5.1–5.5) 9    | 4.9 (4.8–5.0) 9    | < 0.001   |
| MTL          | 5.2 (5.0–5.4) 9    | 4.8 (4.7–4.9) 9    | < 0.001   |
| M1M3         | 2.9 (2.8–3.2) 9    | 2.7 (2.6–2.8) 9    | < 0.001   |
| MAL          | 9.8 (9.5–10.3) 4   | 9.0 (8.8–9.2) 9    | –         |
| MAN          | 5.5 (5.3–5.9) 8    | 5.1 (4.9–5.3) 9    | < 0.001   |

Silky fur (length of dorsal fur 5–6 mm; length of ventral fur 3–4 mm); dorsal fur Dresden-Brown with little contrast between bases and tips slightly lighter tips; ventral fur with blackish bases and Light-Buff tips (Fig. 4A). Specimens of *M. n. larensis* have long silky fur (length of dorsal fur 6–8 mm; length of ventral fur 5–6 mm); dorsal fur strongly bicolored, with blackish bases (2/3) and Tawny-Olive tips (1/3); ventral fur with blackish bases and whitish tips (Fig. 4B). The sagittal crest is absent in *M. n. nesopolus*, the lambdoidal crests are generally absent or very low, and the parietal is inclined forward. Sagittal and lambdoidal crests are present in *M. n. larensis*, ranging from low to moderate in development, and the parietal is not inclined forward. In both populations, the second upper premolar (P3) is aligned in the toothrow and visible in labial view, and the occipital region is always rounded (Fig. 5).
The congruence between the molecular and morphological evidence indicates that the two subspecies of *M. nesopolus* do not form a clade. Thus, *M. larensis* represents an independent evolutionary lineage and should be treated as a full species.
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**Description and comparisons**

*Myotis larensis* is a small-sized bat (total length 78–82 mm; forearm length 31.2–33.2; body mass 3–5 g), morphologically similar to several Neotropical congeners. Ears are moderate in size (length 10–13 mm), and when laid forward extend halfway from eye to nostril. Antitragal notch is barely evident. Membranes are Mummy-brown. Fur on dorsal surface of uropatagium extends slightly past the knees. Plagiopatagium is attached to the foot at toes level by a broad band of membrane. Third metacarpal, tibia, and skull are long in relation to forearm (mean ratios 0.96, 0.48, and 0.43, respectively; see LaVal (1973)).

*Myotis larensis* can be distinguished from all Caribbean and South American congeners by qualitative and quantitative traits. It differs from *M. nesopolus* by its larger size (no overlapping in forearm length and greatest length of skull), presence of sagittal crest, and dorsal fur longer and strongly bicolored. Considering the *Myotis* species

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**Figure 5.** Skull profiles of *Myotis larensis* (AMNH 130709 [holotype]) from Venezuela in lateral (A), ventral (B) and dorsal (C) views; and *Myotis nesopolus* (USNM 105128 [topotype]) from Curaçao in lateral (D), ventral (E) and dorsal (C) views. The image of the *M. nesopolus* skull was inverted.
that occurs in the northern South America, *M. larensis* differs from *M. albescens* (É. Geoffroy, 1806) by the absence of a fringe of hairs along the trailing edge of the uropatagium; from *M. keaysi* J. A. Allen, 1914, *M. pilosatibialis* LaVal, 1973, *M. riparius* Handley, 1960, and *M. simus* Thomas, 1901 by the long silky dorsal fur strongly bi-colored. *Myotis larensis* can also be distinguished from *M. simus* by the plagiopatagium broadly attached at base of the toes. *Myotis larensis* differs from *M. diminutus* Moratelli & Wilson, 2011 by its larger cranial dimensions and dorsal fur strongly contrasting; from *M. handleyi* Moratelli et al., 2013 by its strongly contrasting and long silk dorsal fur and shorter forearm; from *M. oxyotus* (Peters, 1867) by having a smaller skull, less steeply sloping frontals and strongly contrasting dorsal fur. *Myotis larensis* differs from *M. attenboroughi* Moratelli et al., 2017 by its lighter and strongly contrasting dorsal fur and larger skull; and from *M. clydejonesi* Moratelli et al., 2016 by its moderate steeply sloping frontals, less inflated braincase, smaller skull and dorsal fur strongly contrasting. *Myotis larensis* differs from *M. caucensis* Allen, 1914 by its smaller skull and strongly contrasting dorsal fur. *Myotis larensis* can be distinguished from *M. cf. nigricans* from northern South America (sensu Moratelli et al. 2013) by the lighter dorsal and ventral fur, more developed sagittal and lambdoid crests and parietal not inclined forward.

**Discussion**

Genoways and Williams (1979) determined that mainland and island specimens of *M. larensis* and *M. nesopolus*, respectively, were morphometrically similar, with Venezuelan specimens slightly smaller than those from Curacao. As a result, they recognized *M. larensis* as a subspecies of *M. nesopolus*, which was followed by subsequent authors (e.g., Simmons 2005; Wilson 2008; Moratelli et al. 2019b). However, our results do not support this arrangement, indicating a morphometric discontinuity and qualitative morphological differences between *M. larensis* and *M. nesopolus*.

Previous phylogenetic studies based on mitochondrial and nuclear DNA recovered *M. nesopolus* and *M. larensis* as sister lineages and questioned the subspecific status of *M. larensis* because the cyt-b genetic distance of 4% between mainland and Antilles populations suggests a potential for separation at the species level (see Bradley and Baker 2001; Larsen et al. 2012b). However, this study did not include the mainland samples from Ecuador and Peru. Our phylogenetic analyses revealed that *M. nesopolus* and *M. larensis* are not sister lineages and do not share haplotypes. The genetic distances between *M. nesopolus*, *M. larensis* and their sister species are greater than 2%. About this, Bradley and Baker (2001) indicate that genetic distance values between 2 and 11% from cyt-b sequences had a high probability of being indicative of conspecific populations or valid species and merit additional study concerning specific status. Our investigation found a conspicuous phenotypic discontinuity in variation of both the size and shape of the skull and other external characters. Thus, the strong congruence between the morphological, morphometric and molecular evidence presented here supports the hypothesis that *M. larensis* represents a full species.
Nevertheless, it is important to mention the limitation of cyt-b gene for establishing species boundaries in the Caribbean clade, particularly between *M. larensis* and *M.* sp. 1 from Ecuador and between *M. nesopolus* and *M.* sp. 2 from Peru. Although widely used (e.g., Larsen et al. 2012a, b; Moratelli et al. 2016, 2017; Carrion-Bonilla and Cook 2020), the application of cyt-b data to species delimitation and inference of phylogenetic relationships in *Myotis* from the Caribbean clade was insufficient. This demonstrates the need to expand the use of new genetic markers for future systematic studies with the Caribbean *Myotis* assemblage.

With the recognition of *M. larensis* at the species level hierarchy, *M. nesopolus* is restricted to Bonaire and Curaçao and is the only species of the genus found in these islands (Fig. 6). Similarly, other Caribbean islands have unique *Myotis* species, including: *Myotis dominicensis* Miller, 1902 restricted to Dominica and Guadeloupe; *Myotis martiniquensis* LaVal, 1973 is restricted to Martinique; *Myotis attenboroughi* is restricted to Tobago; and *Myotis nyctor* LaVal & Schwartz, 1974 is restricted to Barbados and Grenada (LaVal 1973; Larsen et al. 2012a; Moratelli et al. 2017). However, the taxonomic status of some populations of these species needs to be reassessed. For example, *Myotis nyctor* was described from Barbados and subsequently recorded from Grenada (LaVal 1973; LaVal and Schwartz 1974; Moratelli et al. 2017). Although our phylogenetic analysis grouped the samples of *M. nyctor* from Barbados (N = 5) and Grenada (N = 1) in the...
same clade (Fig. 1), and with low genetic distance between them (ca 0.2%; Table 2), there are qualitative and quantitative morphological differences between specimens from these two islands (see Larsen et al. 2012a). The similarity in the cyt-b sequences between Grenada and Barbados specimens may be explained by the retained ancestral polymorphism due to the very recent separation (Stadelmann et al. 2007; Larsen et al. 2012a).

The biogeographic interpretations made by Larsen et al. (2012b) suggest at least two independent *Myotis* invasions into the Lesser Antilles, and reverse colonization by Caribbean *Myotis* to mainland Central and South America—the latter being a well-documented pattern in other Caribbean bat lineages (Dávalos 2005, 2006, 2010; Genoways et al. 2005; Pavan and Marroig 2017; Tavares et al. 2018). In addition, some biogeographic and ecological aspects suggest the need for taxonomic revision of some species. The distance and geographic isolation between Barbados and Grenada (ca 255 km) are greater than between Dominica and Martinique (ca 42 km), each one having a unique *Myotis* species. Moreover, Barbados and Grenada are separated by the Tobago Basin, with an ocean depth of approximately 2500 m and no ridges that may have connected these two populations during glaciation periods (Speed 1981; Humphrey 1997; Graham 2003). Considering the apparent low vagility and the small home range of *Myotis* in general (e.g., LaVal and Fitch 1977; Castella et al. 2001; Moratelli et al. 2019b), it is possible that the populations of *M. nyctor* from these two islands are isolated and on different evolutionary trajectories. The same rationale might be valid for *M. dominicensis*, where the populations from Guadeloupe and Martinique are isolated by approximately 42 km of sea. However, there are several oceanic ridges between these two islands, which may have served as bridges connecting these two populations during the last glaciation (Speed 1981; Humphrey 1997; Graham 2003). Thus, we suggest that future studies on systematics and biogeography of Caribbean *Myotis* should focus on the definition of the taxonomic status of island populations from Grenada and Guadeloupe.

With the recognition of *M. larensis* as a full species, 28 species of Neotropical *Myotis* (sensu Stadelmann et al. 2007) are currently recognized: *M. albescens* (É. Geoffroy, 1806), *M. ruber* (É. Geoffroy, 1806), *M. nigricans* (Schinz, 1821), *M. levis* (I. Geoffroy, 1824), *M. chiloensis* (Waterhouse, 1840), *M. oxytus* (Peters, 1866), *M. atacamensis* (Lataste, 1892), *M. nesopolus* Miller, 1900, *M. simus* Thomas, 1901, *M. dinelli* Thomas, 1902, *M. dominicensis* Miller, 1902, *M. caucensis* Allen, 1914, *M. keaysi* J.A. Allen, 1914, *M. riparius* Handley, 1960, *M. elegans* Hall, 1962, *M. larensis* LaVal, 1973, *M. martiniquensis* LaVal, 1973, *M. pilosatibialis* LaVal, 1973, *M. nyctor* LaVal & Schwartz, 1974, *M. diminutus* Moratelli & Wilson, 2011, *M. lavali* Moratelli et al., 2011, *M. izecksohni* Moratelli et al., 2011, *M. handleyi* Moratelli et al., 2013, *M. midastactus* Moratelli & Wilson, 2014, *M. clydejonesi* Moratelli et al., 2016, *M. attenboroughi* Moratelli et al., 2017, *M. bakeri* Moratelli et al., 2019, and *M. armiensis* Carrión-Bonilla & Cook, 2020. However, our results indicate that there are at least four haplogroups that might correspond to undescribed species. This scenario confirms the Neotropical region as a highly diverse region for *Myotis*. 
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Appendix 1

List of specimens examined in the American Museum of Natural History (AMNH, New York, USA); Carnegie Museum of Natural History (CM, Pittsburgh, USA); Field Museum of Natural History (FMNH, Chicago, USA), Louisiana State University, Museum of Zoology (LSUMZ, Baton Rouge, USA); Museu de Zoologia da Universidade de São Paulo (MZUSP, São Paulo, Brazil); Museum of Texas Tech University (TTU, Lubbock, USA); Museum of Vertebrate Zoology, University of California (MVZ, Berkeley, USA); National Museum of Natural History, Smithsonian Institution (USNM, Washington, D.C., USA); Natural History Museum of Los Angeles County (LACM, Los Angeles, USA); Natural History Museum, University of Kansas (KU, Lawrence, USA); and Royal Ontario Museum (ROM, Toronto, Canada). Specimens marked with asterisks were included in the morphometric multivariate analysis.

*Myotis albescens* (*N* = 10). Venezuela: Trujillo, Valera, Río Motatán (USNM 370933); Apure, Pto. Páez, Río Cinaruco (USNM 373913); Bolívar, Río Supamo, 50 km SE
El Manteco (USNM 387693); Miranda, 7 km E Río Chico, Nr. Pto. Tuy (USNM 387700); Amazonas, Capibara, 106 km SW Esmeralda, Brazo Casiquiare (USNM 409392, 409395); Amazonas, San Juan, 163 km ESE Pto. Ayacucho, Río Manaipiare (USNM 409403, 409408, 409410, 409411).

Myotis attenboroughi \((N = 13)\). Trinidad and Tobago: Tobago Island, Charlottesiville, 1 km N of Pirate’s Bay, Saint John Parish (USNM 540692 [paratype], 540693 [holotype]); Tobago Island, St. Mary Parish, Hillsborough Reservoir (USNM 538064, 538065, 538066, 538067, 538068, 540619, 540620, 540621, 540694, 540695 [paratypes]).

Myotis caucensis \((N = 22)\): Colombia: Valle del Cauca, Cauca river (AMNH 32787 [holotype]); Valle del Cauca, Candelaria, Ingenio Mayangüez (USNM 461858–461867). Peru: Cuzco, Madre de Dios, 15 km E Puerto Maldonado, Reserva Cuzco Amazónico (KU 144288–144291); Loreto, Yarinacocha (LSUMZ 12252, 12254–12258).

Myotis clydejonesi \((N = 1)\): Suriname: Sipaliwini, Raleigh Falls (TTU 109227 [holotype]).

Myotis diminutus \((N = 2)\): Ecuador: Los Ríos, Santo Domingo, 47 Km S (By Road), Río Palenque Science Center (USNM 528569 [holotype]). Colombia: Nariño, La Guayacana (LACM 18761).

Myotis handleyi \((N = 27)\). Venezuela: Araguá, Rancho Grande Biological Station, 13 km NW Maracay (USNM 517503, 562923, 562924, 562925, 562926–562933, 562934, 562935, 562936, 562937); Distrito Federal, Pico Ávila, 5 km NE Caracas, near Hotel Humboldt (USNM 370932 [holotype]); Distrito Federal, Pico Ávila, 5 km NE Caracas, near Hotel Humboldt (USNM 370891 [paratype]); Miranda, Curupao, 5 km NW Guarenas (USNM 387723); Monagas, 3 km NW Caripe, near San Agustín (USNM 409391, 409429–409431, 409433, 409435, 409437, 409438).

Myotis keaysi \((N = 45)\). Venezuela: Araguá, Rancho Grande Biological Station, 13 km NW Maracay (USNM 370893–370895, 370898–370902, 370911–370913, 370915–370922, 370924, 370926, 370929); Araguá, Rancho Grande Biological Station, 13 km NW Maracay (USNM 370927, 370928, 370930, 370931); Araguá, Pico Guayamayo, 13 km NW Maracay (USNM 521564); Araguá, Rancho Grande, Portachuelo (USNM 562920, 563005, 563006); Araguá, Rancho Grande (USNM 562921); Bolívar, Gran Sabana (USNM 130625, 130626); Carabobo, Montalbán, 4 km NW Montalbán, La Copa (USNM 441741, 441742); Distrito Federal, Los Venados, 4 km NW Caracas (USNM 370889); Distrito Federal, Pico Ávila, 5 km NNE Caracas, near Hotel Humboldt (USNM 370890); Distrito Federal, junction Puerto Cruz Highway and Colonia Tóvar Highway, 0.5 km W (USNM 562984); Guárico, Hacienda El Vira, 10 km NE Altacragua (USNM 387707); Miranda, San Andrés, 16 km SE Caracas (USNM 373920); Miranda, Curupao, 5 km NW Guarenas (USNM 387714–387716, 387718); Monagas, Caripe (USNM 534265).

Myotis larensis \((N = 16)\). Venezuela: Lara, Río Tucuyo (AMNH 130709* [holotype]); Falcón, Capatárida, 6 km SSW (USNM 441710*, 441711*, 441728*, 441735*,
Myotis nesopolus (N = 26). Curaçao: Punda (USNM 101849 [holotype]); Willemstad, Scharloo (USNM 102158); Westpunt, 2.8 km S, 4.5 km E of (CM 52432, 5433*). Bonaire, 8.5 km N, 2 km Wkrandijk (CM 52203, 52204, 52205, 52206, 52207, 52208, 52209, 52211, 52212*, 52213, 52214, 52215, 52216*, 52217*, 52218*, 52219*, 52220*, 52221, 52222*, 52223*, 52224, 52225).

Myotis cf. nigricans (N = 23). Suriname: Para, Zanderij (CM 63933, 69053, 77699).

Venezuela: Carabobo, Urama, 10 Km NW Urama, El Central (USNM 140447, 373921–373924, 373926, 373929, 373932, 373933, 373935, 373936, 373942, 373943, 373946, 373948, 373949, 373950, 441741, 441742).

Myotis oxytus (N = 9). Venezuela: Amazonas, Cerro Duida, Cano Culebra, 50 km NW Esmeralda (USNM 405799); Amazonas, Cerro Neblina, Camp VII (USNM 560809–560811); Bolívar, Km. 125, 85 km SE El Dorado (USNM387712); Bolivar, El Pauji, 21 km NE Icaboru, El Pauji (USNM441750); Distrito Federal, Alto Ñe León, 33 km SW Caracas (USNM 409427); Mérida, La Mucuy, 4 km E Tabay (USNM373919, 387705).

Myotis pilosatibialis (N = 11). Trinidad and Tobago: Trinidad Island, St. George (TTU 5441). Honduras: Francisco Morazán, 1 km W Talanga (LACM 36879 [holotype]). Guatemala: Chimaltenago, Chocoyos (FMNH 41653, 41839, 41840, 41841, 41843, 41844, 41845, 41846, 73365).

Myotis riparius (N = 33). Costa Rica: Puntarenas, 5.3 km S (byroad) San Vito (CM 92491); Limon, Fila La Maquina (LSUMZ 12928). French Guiana: Paracou, near Sinnamary (AMNH 266376, 268591). Guyana: Barima-Waini, North West District (USNM 568021); Potaro-Siparuni, Iwokrama Field Station, Iwokrama Forest (ROM 112049); Potaro-Siparuni, Iwokrama Reserve, Burro Burro River, 25 km WNW of Kurupukari (ROM 107278, 114620); Potaro-Siparuni, Mount Ayanganna, First Plateau Camp (ROM 114688, 114689); Upper Takutu-Upper Essequibo, Gunn’s Strip (ROM 106773). Nicaragua: Chontales (KU 11228). Panamá: Darién, Tacarcuna Village Camp, Río Pucro (USNM 310255 [holotype], 310254, 310256, 310257 [paratypes]); Darién, Río Paya, Mouth (USNM 306798); Panamá, Cerro Azul (USNM 306795); Chiriquí (USNM 331916); Bocas del Toro, Isla Popa, 1 Km SE Deer Island Channel (USNM 464368). Trinidad and Tobago: Trinidad Island, St. George (TTU 5467). Venezuela: Amazonas,Boca Mavaca, 84 km SSE Esmeralda, 7 km up Río Mavaca (USNM 405803, 405804); Amazonas, Capibara, 106 km SW Esmeralda, Brazo Casiquiare (USNM 409457); Amazonas, ca 2 km SE Cerro Neblina Base Camp (USNM 560625); Amazonas, Tamatama, Río Orinoco (USNM 405806); Apure, Nulita, 29 km SW Santo Domingo, Selvas de San Camilo (USNM 416584, 441746, 441748); Aragua, Rancho Grande (USNM 562940); Barinas, 7 km NE Altamira (USNM 441743); Bolívar, Río Supamo, 50 km SE El Manteco (USNM 387721); Bolívar, San Ignacio de Yhuruani (USNM 448544).
The taxonomic status of *Myotis neopolus larensis* is...
| Terminal       | GenBank  | Voucher  | Locality          | Source                                      |
|---------------|----------|----------|-------------------|---------------------------------------------|
| *M. larensis* | JN020569 | TTU 48161 | Guárico, Venezuela | Larsen et al. (2012b)                       |
|               | JX130529 | TTU 48162 | Guárico, Venezuela | Larsen et al. (2012a)                       |
|               | JX130530 | –        | Guárico, Venezuela | Larsen et al. (2012a)                       |
|               | JX130531 | TTU 48163 | Guárico, Venezuela | Larsen et al. (2012a)                       |
|               | JX130532 | TTU 48164 | Guárico, Venezuela | Larsen et al. (2012a)                       |
|               | JX130533 | TTU 48168 | Guárico, Venezuela | Larsen et al. (2012a)                       |
|               | JX130535 | CM 78645  | Guárico, Venezuela | Larsen et al. (2012a)                       |
|               | JX130543 | TTU 48169 | Guárico, Venezuela | Larsen et al. (2012a)                       |
|               | JX130543 | TTU 48169 | Guárico, Venezuela | Larsen et al. (2012a)                       |
| *M. lavali*   | AF376864 | MVZ AD50  | Paraíba, Brazil    | Ruedi and Mayer (2001)                      |
| *M. levis*    | AF376853 | FMNH 141600 | São Paulo, Brazil | Ruedi and Mayer (2001)                      |
| *M. martiniquensis* | AM262332 | –        | Martinique         | Stadelmann et al. (2007)                    |
|               | JN020558 | MNHN:2005–896 | Le Morne–Rouge, Martinique | Larsen et al. (2012b)                       |
| *M. martiniquensis* | JN020557 | MNHN:2005–895 | GrandRivière, Martinique | Larsen et al. (2012b)                       |
|               | JN020561 | –        | GrandRivière, Martinique | Larsen et al. (2012b)                       |
| *M. neopolus* | JN020575 | –        | Bonaire, Netherlands Antilles | Larsen et al. (2012b)                       |
|               | JN020576 | –        | Bonaire, Netherlands Antilles | Larsen et al. (2012b)                       |
|               | JN020577 | –        | Bonaire, Netherlands Antilles | Larsen et al. (2012b)                       |
| *M. nigricans*| JX130450 | TTU 34952 | La Paz, Bolivia    | Larsen et al. (2012a)                       |
|               | JX130528 | TTU 34953 | La Paz, Bolivia    | Larsen et al. (2012a)                       |
|               | JX130545 | TTU 95992 | San Pedro, Paraguay | Larsen et al. (2012a)                       |
|               | JX130496 | TTU 99743 | Presidente Hayes, Paraguay | Larsen et al. (2012a)                       |
|               | JX130499 | TTU 99046 | Alto Paragüai, Paraguay | Larsen et al. (2012a)                       |
|               | JX130539 | TTU 99516 | Concepción, Paraguay | Larsen et al. (2012a)                       |
|               | JX130540 | TTU 99151 | Boquerón, Paraguay | Larsen et al. (2012a)                       |
| *M. nyctor*   | JN020562 | CM 83427  | St. David Parish, Grenada | Larsen et al. (2012b)                       |
|               | JN020563 | TTU 109225 | St. Thomas Parish, Barbados | Larsen et al. (2012b)                       |
|               | JN020564 | TTU 109226 | St. Thomas Parish, Barbados | Larsen et al. (2012b)                       |
|               | JN020565 | TTU 109229 | St. Thomas Parish, Barbados | Larsen et al. (2012b)                       |
|               | JN020566 | TTU 109224 | St. Thomas Parish, Barbados | Larsen et al. (2012b)                       |
|               | JN020567 | TTU 109230 | St. Thomas Parish, Barbados | Larsen et al. (2012b)                       |
| *M. oxyotus*  | AF376865 | FMNH 129208 | Lima, Peru | Ruedi and Mayer (2001)                      |
| *M. pilosatibialis* | JX130449 | TTU 47514 | Yucatán, Mexico    | Larsen et al. (2012a)                       |
|               | JX130525 | –        | Yucatán, Mexico    | Larsen et al. (2012a)                       |
|               | AF376852 | –        | Yucatán, Mexico    | Ruedi and Mayer (2001)                      |
| *M. elegans*  | JX130497 | TTU 84380 | Atlántida, Honduras | Larsen et al. (2012a)                       |
|               | JX130480 | TTU 84138 | Atlántida, Honduras | Larsen et al. (2012a)                       |
| *M. riparius* | AM261891 | –        | La Selva, Costa Rica | Stadelmann et al. (2007)                    |
|               | JX130474 | CM 78659  | Bolívar, Venezuela | Larsen et al. (2012a)                       |
|               | JX130473 | CM 68443  | Para, Suriname     | Larsen et al. (2012a)                       |
|               | JX130469 | TTU 85344 | Esmeraldas, Ecuador | Larsen et al. (2012a)                       |
|               | JX130515 | TTU 85345 | Esmeraldas, Ecuador | Larsen et al. (2012a)                       |
|               | JX130572 | TTU 102681 | Esmeraldas, Ecuador | Larsen et al. (2012a)                       |
|               | JX130492 | TTU 102883 | Esmeraldas, Ecuador | Larsen et al. (2012a)                       |
|               | JX130513 | TTU 84870 | Pastaza, Ecuador   | Larsen et al. (2012a)                       |
|               | JX130506 | TTU 85090  | El Oro, Equador    | Larsen et al. (2012a)                       |
|               | JX130516 | QCAZ 11380 | Chimborazo, Equador | Larsen et al. (2012a)                       |
|               | JX130436 | –        | Huánuco, Peru      | Larsen et al. (2012a)                       |
|               | JX130481 | TTU 46348  | Huánuco, Peru      | Larsen et al. (2012a)                       |
|               | AF376866 | MVZ AD119* | Pernambuco, Brazil | Ruedi and Mayer (2001)                      |
|               | AF376867 | MVZ AD472* | São Paulo, Brazil  | Ruedi and Mayer (2001)                      |
|               | AM262336 | –        | São Paulo, Brazil  | Stadelmann et al. (2007)                    |
|               | JX130485 | TTU 99645 | Paraguari, Paraguay | Larsen et al. (2012a)                       |
|               | JX130486 | TTU 94912  | Canindeyu, Paraguay | Larsen et al. (2012a)                       |
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| Terminal | GenBank | Voucher | Locality | Source |
|----------|---------|---------|----------|--------|
| *M. riparius* | JX130488 | TTU 122454 | Canindeyu, Paraguay | Larsen et al. (2012a) |
| | JX130491 | TTU 99378 | Canindeyu, Paraguay | Larsen et al. (2012a) |
| *M. velifer* | EF222340 | TTU 48587 | Texas, USA | Baird et al. (2008) |
| | EU680299 | TTU 44818 | Texas, USA | Baird et al. (2008) |
| | JX130468 | TTU 109261 | Texas, USA | Larsen et al. (2012a) |
| | AF376870 | MVZ 146766 | Sonora, Mexico | Ruedi and Mayer (2001) |
| | JX130478 | TTU 44816 | Tamaulipas, Mexico | Larsen et al. (2012a) |
| | JX130438 | UAMI 15306 | Michoacán, Mexico | Larsen et al. (2012a) |
| | JX130466 | UAMI 15304 | Michoacán, Mexico | Larsen et al. (2012a) |
| | JX130589 | UAMI 15305 | Michoacán, Mexico | Larsen et al. (2012a) |
| | JX130592 | – | Michoacán, Mexico | Larsen et al. (2012a) |
| | JX130477 | TTU 60983 | Santa Ana, El Salvador | Larsen et al. (2012a) |
| *M. vivesi* | AJ504406 | – | Gulf of California, Mexico | Stadelmann et al. (2004) |
| | AJ504407 | – | Gulf of California, Mexico | Stadelmann et al. (2004) |
| *M. yumanensis* | AF376875 | MVZ 15585 | California, USA | Ruedi and Mayer (2001) |
| *M. sp. 1* | JX130523 | TTU 103803 | El Oro, Ecuador | Larsen et al. (2012a) |
| | JX130541 | TTU 103751 | El Oro, Ecuador | Larsen et al. (2012a) |
| | JX130546 | TTU 102760 | El Oro, Ecuador | Larsen et al. (2012a) |
| | JX130547 | TTU 102765 | El Oro, Ecuador | Larsen et al. (2012a) |
| | JX130548 | TTU 102487 | El Oro, Ecuador | Larsen et al. (2012a) |
| | JX130549 | TTU 102489 | El Oro, Ecuador | Larsen et al. (2012a) |
| | JX130550 | TTU 102490 | El Oro, Ecuador | Larsen et al. (2012a) |
| *M. sp. 2* | JX130452 | TTU 46347 | Huánuco, Peru | Larsen et al. (2012a) |
| | JX130537 | TTU 46344 | Huánuco, Peru | Larsen et al. (2012a) |
| | JX130538 | TTU 46346 | Huánuco, Peru | Larsen et al. (2012a) |
| *M. sp. 3* | JX130493 | TTU 61228 | Valle, Honduras | Larsen et al. (2012a) |
| *M. sp. 4* | JN020570 | CM 63933 | Nickerie, Suriname | Larsen et al. (2012b) |
| | JN020571 | CM 69053 | Para, Suriname | Larsen et al. (2012b) |
| | JN020572 | CM 77699 | Para, Suriname | Larsen et al. (2012b) |
| **Outgroups** | | | | |
| *M. brandtii* | AF376844 | – | Neuhaus, Germany | Ruedi and Mayer (2001) |
| | AM261886 | NMP PB 916 | North west, Russia | Stadelmann et al. (2007) |
| | AY665139 | – | Moscow, Russia | Tsytsulina et al. (2012) |
| | AY665168 | – | Znojmo, Czech Republic | Tsytsulina et al. (2012) |
| *M. gracilis* | AB106609 | – | Hokkaido, Japan | Kawai et al. (2003) |
| | AB243025 | – | Hokkaido, Japan | Kawai et al. (2006) |
| | AB243026 | – | Hokkaido, Japan | Kawai et al. (2006) |
| | AB243027 | – | Hokkaido, Japan | Kawai et al. (2006) |
| | AB243028 | – | Hokkaido, Japan | Kawai et al. (2006) |
| | AB243029 | – | Hokkaido, Japan | Kawai et al. (2006) |
| | AB243030 | – | Hokkaido, Japan | Kawai et al. (2006) |