In search of a meaningful classification for Amazonian marmosets: Should dwarf marmosets be considered *Mico* congenerics?

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The criteria to classify the diversity of Neotropical Primates have recently taken the discussion boards due to the reappraisal on the phylogenetic relationship of some groups. Such controversial and arbitrary decisions, however, can hamper conservation actions in as much as it becomes difficult to prioritise and set meaningful targets. It is the case for dwarf marmosets from central Amazonia. Today, the classification of dwarf marmosets in *Mico* or *Callibella* genus has not been satisfactorily settled. Aiming to contribute to the taxonomic and conservation assessment of dwarf marmosets, we conducted new data collection during 3-year fieldwork in the Aripuanã River, where the species was discovered. We present the first phylogenomic analysis of the evolutionary relationships between marmosets, new data from mitochondrial DNA and morphology, as well new records to clarify geographic distribution. With this new evidence, we support dwarf marmosets as the genus *Callibella*. We further discuss the implications for the conservation of this marmoset.

1 | INTRODUCTION

Since the last two main proposals for the classification of Neotropical primates (Groves, 2001; Rylands et al., 2000), several new classification schemes at the genus level have been presented for some groups (Byrne et al., 2016; Lynch-Alfaro, Silva, & Rylands, 2012; Rylands et al., 2016; Schneider et al., 2012). The reason for such debate is primarily rooted in the

field of molecular phylogenetics, which, in addition to often unveiling a much greater diversity of lineages than initially suspected, provides a timescale on which this diversification occurred. Consequently, divergence time has been widely adopted as an argument to support the classification of lineages of Neotropical primates, following the proposal of Goodman et al. (1998).

However, the use of the divergence time as a key criterion for taxonomic classification in the genus level of Neotropical Primates has been discussed in recent publications (Garbino, 2015a,b; Gutiérrez & Marinho-Filho 2017). Accordingly, divergence time, as well as geographic distribution, is not a diagnostic character and should not be used as the sole criterion in the taxonomic classification.

The recent taxonomic reclassifications at the genus level in Neotropical Primates, however, are based on a robust molecular phylogeny that synthesises a number of ecological, morphological and biogeographical parameters previously identified in the studied groups (see Lynch-Alfaro, Silva, et al., 2012; Buchanan-Smith & Hardie, 1997; Hardie & Buchanan-Smith, 1997; Heymann, 1990; Kobayashi, 1995). The most recent proposals were put forward by Buckner, Lynch Alfaro, Rylands, and Alfaro (2015) and Rylands et al. (2016) for the tamarins (formerly the genus *Saguinus*, currently the genera *Saguinus* and *Leontocebus*) and by Byrne et al. (2016) for the titi monkeys (formerly the genus *Callicebus*, currently the genera *Callicebus*, *Cheracebus* and *Plecturocebus*).

Buckner et al. (2015) presented a phylogenetic and biogeographical analysis that support the splitting of the large- and small-bodied tamarin lineages, proposing the use of a distinct generic name, *Leontocebus* Wagner, 1839, for the *nigricollis* group. Rylands et al. (2016) reviewed the taxonomy of the tamarins and supported previous molecular and biogeographical analyses (Buckner et al., 2015; Matauschek, Roos, & Heymann, 2011), classifying the small-bodied species (*nigricollis* group) in the genus *Leontocebus* Wagner, 1839, and retaining the large-bodied species in the genus *Saguinus* Hoffmannsegg, 1807. In the same way, Byrne et al. (2016) reconstructed the phylogenetic relationships of the titi monkeys and proposed a new genus for the *torquatus* species group (*Cheracebus*) and for the *donacophilus* and *moloch* species groups (*Plecturocebus*), retaining only the group *personatus* in the genus *Callicebus* Thomas, 1903.

Although these authors follow an age-driven classification scheme, other parameters were agued to support those proposals such as the differences in morphology and ecology. An example is the widespread sympatry of species of the *nigricollis* and *mystax* groups (Buckner et al., 2015; Rylands et al., 2016), with members of the two species groups commonly forming mixed groups and using different forest strata to forage (Buchanan-Smith & Hardie, 1997; Hardie & Buchanan-Smith, 1997; Heymann, 1990). In addition, Byrne et al., 2016 argue that the morphology——especially

cranial morphology, body size and pelage (Hershkovitz, 1977; Kobayashi, 1995)——and the sympatric distribution of the *torquatus* and *moloch* groups—explained by its ecological differences—also support their classification in distinct genera (Byrne et al., 2016).

The variation in size and shape of the body and crania of capuchins was used previously to support a taxonomic classification of robust and gracile species in two subgenera (Silva-Jr., 2001), a rank defended by Gutiérrez & Marinho-Filho (2017). Differences in group size, home range, densities and habitat use were identified where gracile (*Cebus*) and robust (*Sapajus*) capuchins are sympatric (Lynch-Alfaro, Silva, et al., 2012). These morphological and ecological differences in robust and gracile capuchins monkeys (Lynch-Alfaro, Silva, et al., 2012) agree with the biogeographical analyses presented in Lynch-Alfaro, Boubli, et al. (2012) to advocate placing species of these two groups into the genera *Sapajus* and *Cebus*, respectively. This taxonomic classification was largely adopted thereafter (Bezerra et al. 2014; Martins et al. 2015; Robinson et al., 2016; Young & Heard-Booth, 2016; Fedigan, 2017). The divergence time between the *Sapajus* and *Cebus* clades was estimated at 6.2 Ma (Lynch-Alfaro, Boubli, et al., 2012) and the widespread sympatry was explained by a rapid diversification during the Pliocene followed by expansion and invasion by the Atlantic Forest *Sapajus* of the Amazon basin, where currently species of the two genera occur in sympatry (Lynch-Alfaro, Boubli, et al., 2012).

The Amazon marmosets (*Mico*, *Cebuella* and *Callibella*) remain among phylogenetically least studied Neotropical Primates, and the classification of this clade is controversial. In the early 1990s, all marmosets (Atlantic Forest + Amazon) were included in the genus *Callithrix*. However, the first molecular studies of Neotropical primates revealed the pygmy marmoset (*Cebuella*, Gray, 1870) more closely related to the Amazon marmosets (Canavez et al., 1999; Chaves et al., 1999; Tagliaro, Schneider, Schneider, Sampaio, & Stanhope, 1997), than to Atlantic forest marmosets *Callithrix* Erxleben, 1777. Thereafter, Rylands et al. (2000) proposed a classification scheme where *Cebuella* was maintained as a valid monophyletic genus for pygmy marmosets and the genus *Mico* Lesson, 1840, was revalidated for the Amazonian marmosets of the "*argentata* group". The Atlantic marmosets, the "*jacchus* group", were maintained in the genus *Callithrix*.

van Roosmalen, van Roosmalen, Mittermeier, and Fonseca (1998) described a "new and distinctive" dwarf marmoset (*Callithrix humilis*) based on the external morphology of an adult male kept as a pet. As the description of van Roosmalen et al. (1998), the classification of this diminutive marmoset is under debate. The first change in classification came about from the proposal of Rylands et al. (2000) with the species reclassified as *Mico humilis*. Three years later, van Roosmalen and van Roosmalen (2003) analysed two additional individuals and for the first time assessed the

phylogenetic relationship of *M. humilis* to other Amazonian marmosets using the mitochondrial control region. The authors concluded that these three dwarf marmosets were distinct enough from *Mico* and *Cebuella* to justify placing them in their own genus: *Callibella*. In the meantime, Aguiar and Lacher (2003) presented a craniometric analysis to reinforce the distinctiveness of dwarf marmosets and to support the classification of *M. humilis* in the genus *Callibella*.

Groves (2001, 2005) adopted an age-related molecular classification based on Goodman et al. (1998) and included *Mico*, *Cebuella* and *Callithrix* and *Callibella* as subgenera of *Callithrix*. Rylands, Coimbra-Filho, and Mittermeier (2009) kept *Callibella* as a genus, following the criteria proposed by Rylands et al. (2000) where *Cebuella* and *Mico* were recognised as distinct genera. Recently, Schneider et al. (2012) combined the mitochondrial control region with four nuclear regions containing *Alu* elements and argued that dwarf marmosets are *Mico* congenerics. Garbino (2015a) also supported the classification of dwarf marmosets as *M. humilis* based on a phylogenetic analysis of a morphological data set.

In fact, the genus-level classification of *Callibella humilis* was based on little material available for analysis and little information from the field. Almost two decades after its description, the classification of dwarf marmosets remains controversial. Here, we presented the first phylogenomic assessment of evolutionary relationship among Amazonian marmosets' genera and a reappraisal of the classification of dwarf marmoset arguing for the use of the genus *Callibella*. We included an entirely new data set obtained through fieldwork and present the following information in our analysis:

(i) skull morphology, (ii) body size and (iii) updated geographic distribution and the overlap to *Mico marcai*. In addition, we provided an important start point for its conservation assessment based on threats and phylogeny by calculating its EDGE score (Isaac, Turvey, Collen, Waterman, & Baillie, 2007).

2 | MATERIALS AND METHODS

2.1 | Surveys

In this study, we conducted five field expeditions between January 2012 and February 2015 in the Marmelos–Aripuanã interfluve (Figure 1). Data collections were carried out using existing trails. We recorded the number of individuals sighted in each group of dwarf marmosets found, and if they were associated with other primates. In 2015, we opened 10 transects in upland forests with an average length of 3.07 ± 0.63 km and 1 m width keeping a minimum distance of 2 km between adjacent transects. We placed the transects randomly in the study areas, totalling an effort of 271.6 km surveyed. The transects were travelled twice a day by two observers moving at a speed of 1.5 km/hr, during the early morning from 7 to 11 hr and the afternoon from 14 to 17 hr. We defined an interval of two paused days after travelling each transect to reduce the impact of the observers' presence on the primates' behaviour. When a group of marmosets was detected, we counted the number of individuals to estimate the encounter rate. We present new records of dwarf marmosets (*C. humilis*) and compare the sighting rate with the sympatric Marca's

FIGURE 1 The records of *Callibella humilis* in the literature and in this study. The dark grey area is the estimated geographic range of the species considered in the last assessment of the conservation status
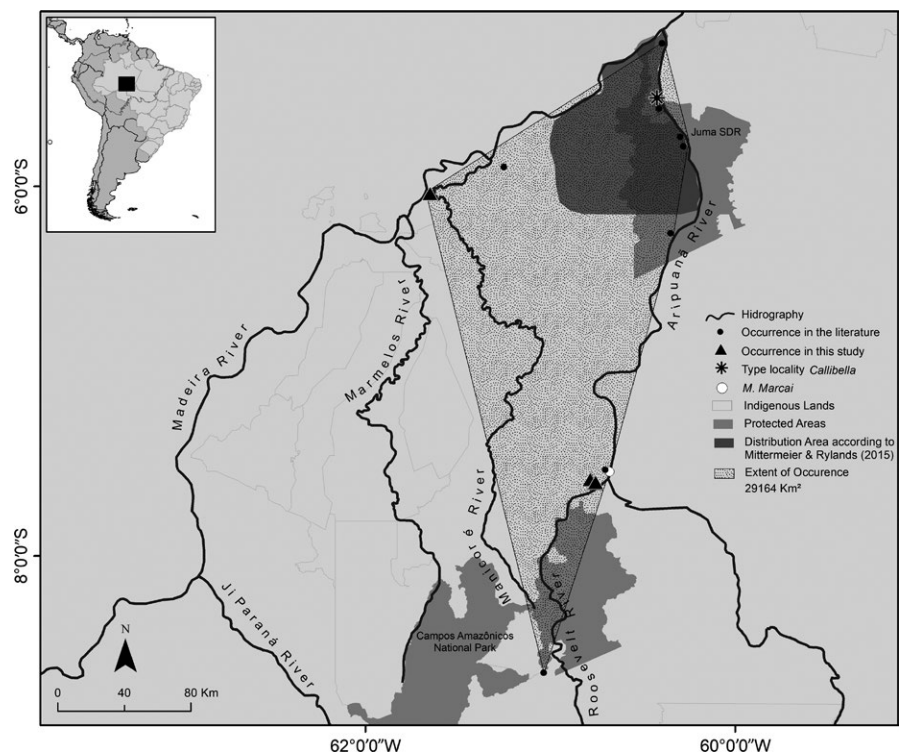
marmoset (*Mico marcai*). Finally, we estimate the extent of occurrence based on the new data collected in this study.

2.2 | Morphometrics

We examined 104 specimens (skins and skulls) from scientific collections, including 16 specimens recently collected in a broad study of the taxonomy, biogeography and conservation of marmosets from Aripuanã River basin (Silva, Nunes, & Bastos, 2013; permit SISBIO numbers 13507 and 6493-1). For each collected specimen, we recorded sex, age category, body mass and standard mammalian measurements (head-body length, tail, foot and ear) in the field. The specimens were stored in the mammalian collection of the Museu Paraense Emílio Goeldi (MPEG), Belém, Brazil.

Only adult specimens, determined by the complete erupted dentition and by the complete fusion of the spheno-occipital and sphenoethmoidal sutures, were used for craniometrics. We used digital callipers (precision 0.01 mm) to obtain craniometric variables following Hershkovitz (1977). We analysed the data using a principal component analysis in R 3.3.3 (R Development Core Team, 2017). This analysis captures the multidimensionality of the cranium measurements and reduces it into few principal components, that is, axis. Here, we used the first two axes to represent the cranium metrics of specimens in two dimensions to test whether there are discrete morphological groups that correspond to the different marmoset genera. Additionally, we present an anatomical description of the skull of *C. humilis* and compared with its sympatric Marca's marmoset (*M. marcai*).

2.3 | Molecular analyses

For molecular phylogenetic analyses, we extracted whole genomic DNA from tissue samples using standard phenol–chloroform extraction protocol of Sambrook, Fritsch, and Maniatis (1989). We amplified the complete mitochondrial cytochrome b gene by polymerase chain reaction (PCR) with the primers MonkeyGluF1 (5′-CCATGACTAATGATATGAAAARCC-3′) and MonkeyProR1 (5′-AGAATSTCAGCTTTGGGTGTTG-3′; Boubli et al., 2018). PCR products were purified using ExoSap (Werle, Schneider, Renner, Volker, & Fiehn, 1994) and subjected to fluorescent dye-terminator (ddNTP) sequencing following the manufacturer's recommended protocol for BigDye sequencing chemistry (Applied Biosystems) and using the primers MonkeyCytbF2 (5′-GGATCAARYAAYCCRTCAGG-3′), MonkeyCytbR1 (5′-GCBCCTCAGAADGATATTTG-3′) and MonkeyCytbR2 (5′-CGTAGRATTGCRTATGCRAA-3′; Boubli et al., 2018). Subsequent to the cycle sequencing reaction, the products were precipitated with 100% Ethanol/125 mM EDTA solution, resuspended in Hi-Di formamide and resolved on an ABI 3130xl automatic sequencer (Applied Biosystems). Sequences were assembled, edited, aligned and trimmed using the software Geneious v8.1.8; alignment was made using MUSCLE (Edgar, 2004) plug-in and conferred visually.

We also performed a partial representational genome sequencing using the double-digest RAD sequencing protocol (ddRAD; Peterson, Weber, Kay, Fisher, & Hoekstra, 2012). The standard protocol was adapted to allow simultaneous digestion and adaptor ligation, and for use on the Ion Torrent PGM (https://github.com/legalLab). Briefly, 200 ng of genomic DNA of each individual was digested with SdaI and Csp6I restriction enzymes (Fermentas) and the Ion Torrent P and A adapters were linked to the digested fragments, all in one step. The fragments were enriched via PCR. The A adaptor is a "Y divergent" (Coyne et al., 2004), resulting in the enrichment of only those ddRAD fragments with one P1 and one A adaptor. Furthermore, the A adaptor contains a unique molecular barcode for identification of individuals. Following the PCR enrichment, we selected fragments in the range of 320–400 bp using the Pippin Prep (Sage Science). Based on the analysis of *complete* primate genomes deposited in Genbank, we expected to observe ~12,000 ddRAD fragments in the range of 320–400 bp. This information was then used to optimise the number of individuals to be analysed in each run of the Ion Torrent PGM. The complete ddRAD protocol, scripts for estimating the number of ddRAD fragments in a given size range and scripts for extracting ddRAD fragments from published genomes are available on GitHub (https://github.com/legalLab).

To construct our phylogenetic trees, we included fresh or dried tissues from *C. humilis* (n = 2 [5]), *Cebuella* cf. *niveiventris* (n = 1), *Cebuella* aff. *pygmaea* (n = 1), *Mico argentatus* (n = 1), *M. humeralifer* (n = 1), *M. intermedius* (n = 1), *M. marcai* (n = 1), *M. mauesi* (n = 1) and *Callithrix jacchus* (n = 1); *Callimico goeldii* (n = 1) and *Saguinus bicolor* (n = 1) were included as outgroups. For mitochondrial DNA analyses, we collected 1,140 bp of the cytochrome *b* gene. For phylogenomic analyses, we analysed 340,593 nucleotides representing 1,063 loci. Bayesian time tree was estimated in the program BEAST v2.4.2 (Drummond, Suchard, Xie, & Rambaut, 2012) via constraining the divergence of *Saguinus bicolor* from the callitrichid clade at 14.89 mya and *Callimico goeldii* from other callitrichids at 10.68 mya. We assumed normally distributed secondary calibrations, with means and standard deviations of divergence times obtained from (Perelman et al., 2011). We also estimated phylogenetic relationships within the maximum likelihood framework implemented in RAxML (Stamatakis, 2014) and the Bayesian framework implemented in ExaBayes (Aberer, Kobert, & Stamatakis, 2014).

2.4 | EDGE analyses

Evolutionary Distinct, Globally Endangered (EDGE) analyses (Isaac et al., 2007) were carried out using a custom script

written in the statistical language R (R Development Core Team, 2017). We calculated the evolutionary distinctness (ED) score using the ecol.distinct function in the package picante (Kembel et al., 2010) with the ddRADseq phylogeny estimated in BEAST as input. EDGE scores for each taxon were then calculated using formula (1) of Isaac et al. (2007) and the current Red List extinction risk category of each taxon.

3 | RESULTS

3.1 | Surveys

We detected nine groups of *C. humilis* with a total of 18 individuals (mean = 2.0 individuals/group, *SD* = 1.12). The number of individuals sighted per group ranged from 1 to 5, and the estimated encounter rate was 0.033 groups/km and 0.066 individuals/km. Comparatively, the number of sighted individuals per group of the sympatric *Mico marcai* ranged from 1 to 11 (mean = 4.56 individual/group, *SD* = 2.42, *N* = 41), in an encounter rate of 0.11 groups/km and 0.50 individuals/km. Both species were found travelling together on two situations; however, it is unclear if they forage in mixed groups. We had three other occasional records of *Callibella humilis* in this area. The localities where we found the dwarf marmosets represent a range extension of its distribution (Figure 1). On the left bank of Aripuanã River, we recorded the species close to the mouth of Roosevelt River (see Garbino, Silva, & Davis, 2013) in a secondary forest limited by cassava crops. On this occasion, FES was looking for evidence of *Mico marcai* using a recording of long calls of *Mico emiliae*. An adult male of *C. humilis* came straight towards the researcher while issuing long calls. The other sightings in this region were in a secondary forest between the lower Roosevelt River and the BR-230 Transamazônica highway. We also recorded *C. humilis* on both banks of the lower Manicoré River (Figure 1). Our records, thus, extend the range of *C. humilis* to the left bank of Manicoré River, but further surveys will clarify its presence throughout the interfluve Manicoré–Marmelos. We have no records of the species south of the Campos Amazônicos National Park, but further surveys in the south of this region will clarify if, in fact, the Savanna vegetation of this region delimits the southern occurrence of this species (see Garbino et al., 2013). The extent of occurrence was estimated in 29,164 km².

3.2 | Morphological analyses

Principal component analysis of the 11 craniometric characters clearly differentiates the three genera of Amazon marmosets—*Cebuella*, *Callibella* and *Mico*—from each other

(Figure 2, Table S1). The first two components explained 96.5% of the total variation of the data, with most of this variation explained by the first axis (95.4%). Each cluster contains only allopatric species. The sympatric *Callibella* and *Mico* are clearly discriminated along the first principal axis of the morphospace, while less differentiation is evident between the allopatric *Callibella* and *Cebuella* along both the first and second principal components (Figure 2, Table S2). The species of the genus *Mico* broadly overlap in morphospace (Figure 2); therefore, the majority of variance in shape and size is partitioned between the genera *Callibella* and *Mico* rather than among species within the genus *Mico*. Comparison of specific features of skull anatomy of *Callibella humilis* and *Mico marcai*, two sympatric marmosets, therefore, reflects differences in anatomical features of the supraspecific taxa *Callibella* and *Mico* (Figure S1).

The surface of the parietal bone of *M. marcai* has a lower convexity, especially in the areas of the occipital and frontal angles, giving a flatter appearance (Figure S1). In *Callibella*, a muscular line clearly delimited the middle third of the surface of the parietal bone. This line is positioned in a caudal–rostral direction, being continuous and rougher in the frontal bones (Figure S1). In both species, a temporal line reaches the super ciliary arch marking the point of insertion of the temporalis muscle (Figure S1). This muscle is smaller in *Callibella* and covers approximately 2/3 of the parietal bone and a small portion of the lateral surface of the frontal bone. In *Mico marcai*, the same muscle occupies virtually the entire parietal bone and nearly half the lateral dorsal surface of the frontal bone. The external occipital protuberance, positioned in the middle third of the occipital bone, is wider
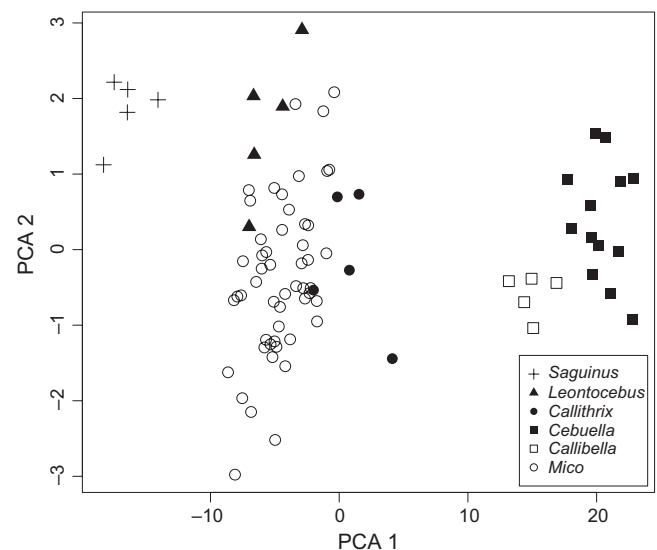

FIGURE 2 Plots of scores for the first two principal components from principal component analysis of cranial variables in Callitrichinae

and prominent in *M. marcai* and more longilineus laterally in *Callibella* (Figure S1).

3.3 | Molecular analyses

In the time tree phylogenomic analyses, *Callibella* and *Mico* were sister taxa with 100% posterior probability and an estimated 2.37 mya divergence; the age of the root of *Mico* was estimated at 1.30 mya (Figure 3). Similarly, in the analyses of the mitochondrial cytochrome b gene, *Callibella* and *Mico* were sister taxa with 100% posterior probability and an estimated 2.33 mya divergence; the age of the root of *Mico* was estimated at 1.05 mya (Figure S2). The same set of highly supported relationships was observed in the maximum likelihood (RAxML) and Bayesian inference (ExaBayes) analyses. The cytochrome b and ddRADseq analyses differed in the relationships of species of the genus *Mico*; however, the sister taxon relationship of the discordant individual—*M. intermedius* FES09—to the clade comprising *M. humeralifer* and *M. mauesi* is poorly supported.

3.4 | EDGE analyses

The Evolutionary Distinct, Globally Endangered (EDGE) score (Isaac et al., 2007) for *Callibella humilis* was 3.30, while EDGE scores for species of the sister genus *Mico* varied from 0.80 to 1.05. Only *Callimico goeldi* had a greater EDGE score at 4.70.

4 | DISCUSSION

4.1 | The criteria for the classification of Amazon marmosets

The divergence times estimated for the three Amazon marmosets clades are smaller than the majority of intergeneric divergences in Neotropical primates (Byrne et al., 2016; Goodman et al., 1998; Lynch-Alfaro, Silva, et al., 2012; Rylands et al., 2016). *Callibella* diverged from *Mico* approximately 2.3 mya, while the main in-group diversification of the extant *Mico* species was estimated in 1.3 mya. However,
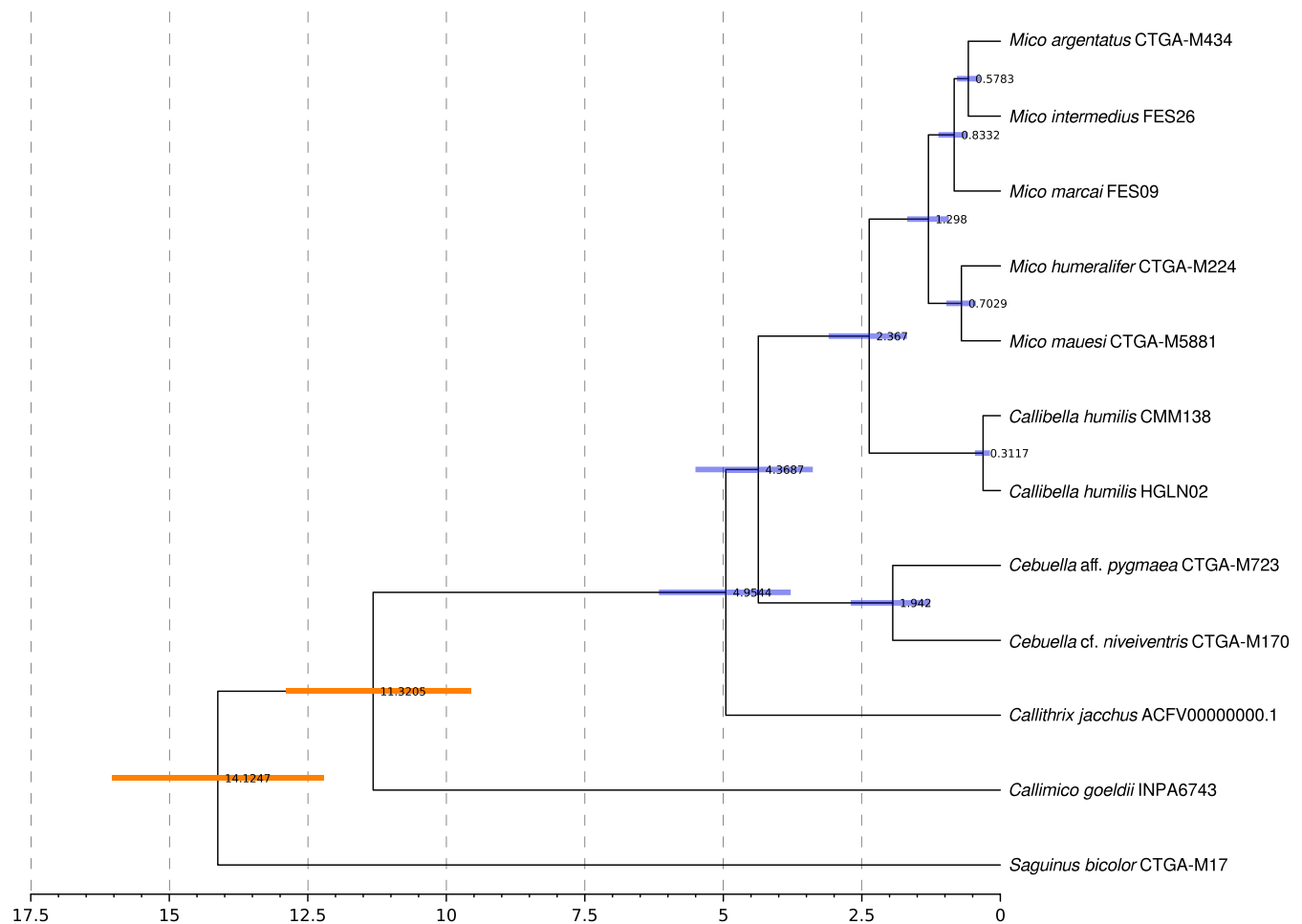

FIGURE 3 Phylogenomic trees obtained through maximum likelihood and Bayesian inference of nuclear DNA (DDRadseq) from *Callibella*, *Mico*, *Callithrix* and *Cebuella* species. Each external branch represents an individual, and the support probability value is given on each branch [Colour figure can be viewed at wileyonlinelibrary.com]

the estimated 1 million years separating the divergence of *Callibella* and *Mico* ancestors, and the beginning of diversification of *Mico*, together with morphological differences between them—both in size and shape—reinforces the distinctness of *Callibella*.

Callibella humilis has a much smaller and lighter body than in *Mico* species and only slightly larger and heavier than *Cebuella pygmaea*, the smallest Neotropical Primate (see Table S2). In addition, *C. humilis* also live in smaller social groups and apparently has a lower density when compared with its sympatric *Mico marcai*. In primates, differences in morphology are associated with differences in resource use, that is, morphology reflects niche use (ecomorphology; Bicca-Marques, 1999; Meloro et al., 2013; Rosenberger, 1992). While niche occupation of *C. humilis* and *M. marcai* remains largely uncharacterised—both species are widely sympatric—their sympatry must be made possible by minimal realised niche overlap. Similarly, to other Neotropical Primates currently classified as different genera (e.g., *Leontocebus* and *Saguinus*; *Cheracebus* and *Plecturocebus*), character displacement minimising realised niche overlap, and thus, the interspecific competition would be a process that reinforces the divergence between *Callibella* and *Mico*.

Since the late 1990's, the classification of marmosets has been in flux, in part due to lack of or conflicting phylogenetic hypotheses and in part due to differing philosophical approaches to classification. In 1998, *C. humilis* was described as a species of the genus *Callithrix*. This was prior to the proposal by Rylands et al. (2000) that the generic name *Mico* is used for Amazonian marmosets found east of the Madeira River and that the pygmy marmosets found west of the Madeira River be placed in the genus *Cebuella* (Canavez et al., 1999; Chaves et al., 1999; Tagliaro et al., 1997). In 2003, van Roosmalen and colleagues reanalysed the data of Tagliaro et al. (1997) including sequence data of "*Callithrix*" humilis. "*Callithrix*" humilis was found to be the sister taxon to the genera *Cebuella* + *Mico*; thus, the authors reclassified "*Callithrix*" humilis in the genus *Callibella*. However, using mitochondrial and nuclear DNA, Schneider et al. (2012) argue for the inclusion of *C. humilis* in the genus *Mico*. The authors based its decision on an estimated time of divergence of slightly less than 2.5 mya between *C. humilis* and other species of the genus *Mico*, which is more recent than the age of the root of diversification of the genera *Callithrix* or *Saguinus*. This argument effectively follows the proposal of Goodman et al. (1998), who suggest that taxa sharing a last common ancestor <4 mya should be classified as members of the same genus.

However, strict divergence time-based criteria, such as that proposed by Goodman et al. (1998), do not convey any other information besides divergence time. We are of the opinion that the genus can and should convey other evolutionary information such as morphological and/or ecological

divergence/distinctness, and as such become the first identifiable entities in biodiversity studies (Dubois, 1988; Vences, Guayasamin, Miralles, & de La Riva, 2013). Vences et al. (2013) pointed out that the attempts to fit inherent biological meaning for taxa of the same supraspecific Linnaean ranks are unrealistic; instead, only species can be considered equivalent to each other because they correspond to the same level of biological organisation. The authors then suggest that an adopted classification scheme should also bring the information that will facilitate the communication between scientists and between those and the lay public.

The generic name is an irreplaceable part of the Latin binomial attributed to all species in the Linnaean binomial, being the first identifiable entity (Dubois, 1988; Vences et al., 2013). The few publications on the genus concept (Dubois, 1988; Garbino, 2015b; Lemen & Freeman, 1984; Talavera, Lukhtanov, Pierce, & Vila, 2013) in contrast to the vast body of literature focusing on species and species concepts indicate how imperative is the discussion of a classification system that best conveys the evolutionary history of a higher-level taxon.

Thus, while the *Callibella* and *Mico* lineages began to diverge at approximately 2.3 mya (Figure 3), which is less than the minimal 4 mya divergence suggested for the diverge of genera by Goodman et al. (1998), the *Callibella* and *Mico* lineages are morphological and ecologically distinct and divergent. The molecular phylogeny in line with the morphological and ecological distinctions supported the classification in different genera of previously congeneric species occurring in sympatry (Byrne et al., 2016; Lynch-Alfaro, Silva, et al., 2012; Rylands et al., 2016). In Neotropical primates, sympatry occurs between species belonging to different genera, where species of the different genera are characterised by different morphologies and different ecological requirement (Ferrari & Martins, 1992; Rylands, 1989). Our morphological analyses support the findings of Aguiar and Lacher (2003, 2009) in that *C. humilis* is divergent and distinct from species of the genus *Mico*. As such, the sympatric distribution between *C. humilis* and *M. marcai* is probably possible due to differences in their realised niche, as found in other Neotropical Primates recently classified in different genera (Byrne et al., 2016; Lynch-Alfaro, Silva, et al., 2012; Rylands et al., 2016). However, several issues should be investigated to clarify this assumption, such as feeding ecology, home range, use of the forest strata and formation of mixed-species group. Considering this evidence and adhering to the school of thought that genus should convey information on the evolutionary history in the higher-level taxon, we propose the following classification for the marmosets:

1—*Callibella* van Roosmalen and van Roosmalen, 2003: as a monotypic genus occurring east of the Madeira River in the Marmelos–Aripuanã interfluve, its area of occurrence

apparently being entirely within the distribution area of *Mico marcai*.

2—*Mico* Lesson, 1840: with 13 valid species occurring east of the Madeira River in the Madeira–Tocantins interfluve, with *M. melanurus* also occurring in the Bolivian basin (headwaters of the Madeira River).

3—*Cebuella* Gray, 1823: a monotypic genus occurring west of the Madeira River. *Cebuella pygmaea* is morphologically similar to *C. humilis* (Figure 2) and is sister to the *Callibella* + *Mico* clade having diverged from the ancestor of this clade at approximately 4.3 mya (Figure 3).

4—*Callithrix* Erxleben, 1777: with six valid species, all occurring in the Atlantic Rainforest. Species of *Callithrix* are morphologically similar to those of *Mico* (Figure 2). *Callithrix* is the sister taxon to the clade comprising the three Amazonian genera (*Callibella*, *Mico* and *Cebuella*) and diverged from them approximately 4.9 mya (Figure 3).

4.2 | The conservation of dwarf marmosets (*Callibella humilis*)

Our field surveys revealed that dwarf marmosets are not restricted to a tiny area between the mouths of Aripuanã and Manicoré Rivers as argued by van Roosmalen & van Roosmalen, 2003). These primates can be found further west, on the left bank of the Manicoré River, and further south, on the left margin of lower Roosevelt River. Our surveys point to a complete overlap of the distribution of *C. humilis* and *M. marcai* (Silva et al., in prep.), with an extent of occurrence at least ten times as large as the area suggested by van Roosmalen and van Roosmalen (2003).

Callibella humilis was confirmed in only two legally protected areas: Juma Reserve of Sustainable Development and Campos Amazônicos National Park. The first has 590,000 ha, delimiting an area on both banks of the Aripuanã River; however, the species is presented only in the sector of the left bank of Aripuanã River. The second protected area in Aripuanã–Marmelos interfluve is the Campos Amazônicos National Park, but just a small northern portion of this park encompassed the dwarf marmoset's distribution. Most of the vegetation in that region is composed of open Cerrado patches with *Campinaranas*—a dense low canopy forest grown over weathered sandy soils. It is unlikely that *C. humilis* will occur in this habitat; thus, the northern portion of the Campos Amazônicos National Park most likely represents the southern distributional limit for *C. humilis*.

Assessing the conservation status of *C. humilis* is a challenge, although an imperative matter that urges at least two stages: systematic surveys and threat assessment. The only indirect inference of its population was provided by van Roosmalen and van Roosmalen (2003) based on the home range size and group size. However, in our surveys, the difficulty to sighting the species in the dense forest because of its diminutive size and cryptic behaviour resulted in the low encounter rate of *C. humilis* (0.066 individuals/km). We suggest, therefore, the use of combined methods, especially linear transects and playbacks, to increase the number of sightings of dwarf marmosets in the wild (Gestich, Caselli, Setz, & Rogério, 2016; Plumptre, Sterling, & Buckland, 2013) to get a reliable estimate of its abundance and density.

In the last IUCN assessment (2015, https://doi.org/10.2305/iucn.uk.2015-1.rlts.t41584a70616233.en), *Callibella humilis*—listed as *Mico humilis* apud Schneider et al. (2012)—was evaluated as Vulnerable D2. The Conservation status of *Callibella humilis* was considered as Least Concern in the national assessment under the justification that there is no evidence of any major threats (Röhe, 2015). In our opinion, the arguments for listing *C. humilis* as Least Concern do not reflect the real conservation status of the species, or are, at least, premature. We defend a careful categorisation based on further population and occurrence data. Considering the current IUCN category for *C. humilis*, its EDGE score (3.30) is the second highest ranked for Amazonian primate after *Callimico goeldii*, with only 18% of all other mammals having higher EDGE score. *Callibella humilis* is a unique taxon as manifested by its phylogenetic uniqueness and its morphological distinctness. As such, the species and its habitat are worthy of focused conservation efforts.

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