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Pelage Variation and Morphometrics of Closely Related *Callithrix* Marmoset Species and Their Hybrids

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Abstract

Background: Hybrids are expected to show greater phenotypic variation than their parental species, yet how hybrid phenotype expression varies with genetic distances in closely-related parental species remains surprisingly understudied. Here we study pelage and morphometric trait variation in anthropogenic hybrids between four species of Brazilian *Callithrix* marmosets, a relatively recent primate radiation. Marmoset species are distinguishable by pelage phenotype and level of morphological specialization for eating tree exudates. Here, we (1) describe qualitative phenotypic pelage differences between parental species and hybrids; (2) test whether significant quantitative differences exist between parental and hybrid morphometric phenotypes; and (3) determine which hybrid morphometic traits show heterosis, dysgenesis, trangression, or intermediacy relative to the parental trait. For morphometric traits, we investigated both cranial and post-cranial traits, particularly as most hybrid morphological studies focus on the former instead of the latter. Finally, we estimate mitogenomic distances between marmoset species from previously published data.

Results: Hybrid facial and overall body pelage variation reflected coloration and patterns seen in parental species. In morphometric traits, *C. jacchus* and *C. penicillata* were the most similar to each other, while *C. aurita* was the most distinct, and *C. geoffroyi* trait measures fell between these other species. Most traits in *C. jacchus* x *C. penicillata* hybrids showed either heterosis or were intermediate relative to the parental trait values. We observed heterosis and dygenesis in traits of *C. penicillata* x *C. geoffroyi* hybrids. Trangressive segregation was observed in hybrids of *C. aurita* and the other species. These hybrids were also *C. aurita*-like for a number of traits. Genetic distance was closest between *C. jacchus* and *C. penicillata* and farthest between *C. aurita* and the other species.

Conclusion: We attributed significant phenotypic differences between marmoset species to differences in morphological exudivory specialization in these species. Our results suggest that intermediate hybrid traits relative to the parental trait values are more likely in crosses between species with relatively lesser genetic distance. More extreme phenotypic variation is more likely in parental species with greater genetic distance, with transgressive traits appearing in hybrids of the most genetically distant parental species. We further suggest that that less developmental disturbances can be expected in hybrids of more recently diverged parental species.

Keywords: Brazil; hybridization; anthropogenic; heterosis; dysgenesis; transgressive segregation; pelage

Background

Hybridization occurs under both natural and anthropogenic contexts, with the former occurring in about 10% of animal species [1], and with the latter increasing between previously isolated populations [2, 3, 4]. Our understanding of the genomic consequences of animal hybridization has grown considerably (e.g.[4, 5, 6, 7]), and the range of hybridization outcomes include but are not limited to hybrid speciation, genetic swamping, adaptive introgression, or extinction [5, 6, 7]. Hybridization also impacts morphological traits [8, 9, 10]. Studies of hybrid morphology to date have largely focused on craniofacial features, but we still possess knowledge gaps in

how hybridization manifests itself in post-cranial anatomy [10]. Most animal hybrid morphology studies also feature a single pair of parental species and the resulting hybrids (e.g. [8, 11, 12, 13, 14, 15, 16]), but there is also interest in understanding how the hybrid phenotype varies with the genetic distances between closely-related parental species [8, 17].

Hybrids are expected to show a more variable array of morphological phenotypes than their parental species [8, 18]. Hybrids can resemble one of their parental species, either in terms of a single trait or as a whole, can be heterotic or dysgenetic relative to the parents (measured as positive or negative deviation from a mid-point value), or can display transgressive traits (i.e. outside of the range of parental variation)[8, 18, 19]. The cumulative effects of gene interactions (dominance and epistasis), parental species temporal divergence, and allele frequency differences between parental species are all thought to underlie morphological phenotypic variation in hybrids [18]. Intermediate traits are explained by a standard polygenic model with additive effects, which is expected for species with small allele frequency differences [8, 18]. However, isolated parental populations with different fixed alleles are expected to produce heterotic hybrids [8, 18]. Dysgenesis is predicted for more distantly related taxa and represents a breakdown of 'coadapted gene complexes' between the parental species [8, 18]. Transgressive traits seem to be related to complementary gene action of antagonistic quantitative trait loci [20, 21].

One key study which looked at the phenotypic effects of hybridization in pairs of parental species within a wide range of genetic distance was conducted experimentally on cichlid fish [17], and there was a particular interest in transgressive traits in this work. In F1 hybrids, the relationship between the frequency of transgressive segregation and level of parental species genetic difference was "bowl shaped," while in F2 hybrids the amount of hybrid transgression increased linearly with parental species genetic distance [17]. However beyond such work, hybrid expression of morphological traits across interbreeding species with variable genetic difference, particularly in non-experimental animal populations, remains understudied.

Primates are one animal group where hybridization is estimated to occur among 7–10% of species [22], and the recent radiation of Brazilian *Callithrix* marmosets makes an excellent model for characterizing the hybridization effects on phenotype in closely-related, interbreeding species. The two phylogenetic subgroups that compose the *Callithrix* genus, the "*aurita*" group (*C. aurita* and *C. flaviceps*) and the "*jacchus*" group (*C. kuhlii*, *C. geoffroyi*, *C. jacchus*, *C. penicillata*), diverged about 3.5 million years ago (Ma) [23]. Within the *jacchus* group, *C. jacchus* and *C. penicillata* are the most recently diverged at 0.51 Ma, followed by *C. kuhlii* at 0.82 Ma, and *C. geoffroyi* at 1.18 Ma [24]. *Callithrix* species are distinguishable from each other based on level of morphological specialization for eating tree gums and exudates (ie. exudivory), facial and overall body pelage patterns and coloration, and peri-auricular ear-tuft shape and color [24]. While natural hybridization occurs between certain pairs of *Callithrix* species under secondary contact at species range boundaries, anthropogenic hybridization has dramatically increased between several species over the last few years as a result of the illegal pet trade [23, 24, 25].

Thus far, most studies of hybrid *Callithrix* phenotypes are based on qualitative descriptions of pelage differences between hybrids and their parental species [26, 27,

28, 29, 30, 31]. Only Fuzessy et al. [32] have tested theoretical expectations of hybrid phenotypic diversity in *C. geoffroyi* and *C. penicillata* hybrids. Here, we build upon these previous studies by examining cranial and post-cranial metric variation among four marmoset species (*C. aurita*, *C. jacchus*, *C. geoffroyi*, *C. penicillata*) along with their hybrids in individuals sampled in the wild or in captivity. Our study represents the largest marmoset morphological sampling to date in terms of hybrid sample number and types of hybrids. We also provide detailed descriptions of several species and hybrid pelage phenotypes. Our main study aims are to: (1) describe qualitative pelage phenotypic differences between parental species and hybrids; (2) test whether significant quantitative differences exist between parental and hybrid marmoset phenotypes; and (3) quantify whether and how hybrid phenotypic variation differs relative to parental species (i.e., intermediate, heterotic, dysgenic, or transgressive). We also estimated genetic distances between our four marmoset species of interest from previously published mitogeomic data that include a subset of our samples [23]. This estimate allowed us to further investigate how aims 2 and 3 vary with differential parental species' genetic distance.

Methods

Sampling

Our samples consisted of 209 adult individuals from four *Callithrix* species (*C. aurita, C. geofforyi, C. jacchus, C. penicillata*) as well as several hybrid types (*C. aurita* x *Callithrix sp.*, *C. penicillata* x *C. geoffroyi*, *C. penicillata* x *C. jacchus*, *C. geoffroyi* x *Callithrix* sp). Samples are summarized by taxon in Table 1 and a detailed list is given in Supplementary Table S1. Following Yamamoto [33] observations of dental characteristics and genitalia growth in marmosets, animals between 5 and 10 months old were classified as juveniles, while those older than 11 months were considered adults. We excluded all non-adult individuals from the phenotypic and morphological analyses described below.

Marmosets were sampled between 2015 and 2019 as follows: (1) wild marmosets in Bahia, Espírito Santo, Minas Gerais, Rio de Janeiro, Pernambuco, and São Paulo states; (2) captive-born, wild-caught, and confiscated marmosets housed at the Guarulhos Municipal Zoo, Guarulhos, São Paulo, CEMAFAUNA (Centro de Manejo de Fauna da Caatinga), Petrolina, Pernambuco, CPRJ (Centro do Primatologia do Rio de Janeiro), Guapimirim, Rio de Janeiro, Parque Ecológico do Tietê (PET), São Paulo, SP, and Divisão Técnica de Medicina Veterinária e Manejo da Fauna Silvestre (DEPAVE-3), São Paulo, SP; (3) a wild group from Natividade, Rio de Janeiro that was caught and housed at CPRJ; and (4) a wild group from Ilha D'Agua, Rio de Janeiro, RJ housed at SERCAS (Setor de Etologia aplicada à Reintrodução e Conservação de Animais Silvestres), Campos dos Goytacazes, RJ. Sampling information and locations of marmosets are described in Table 1, Supplementary Table S1, and Figure 1. Marmoset capture methodology has been described elsewhere [31]. All individuals were allowed to recover after sample collection, and wild marmosets were released at their original point of capture.

Phenotyping

Using the approach developed in Fuzessy et al. [32], marmoset facial markings and pelage characteristics were used to phenotypically differentiate between species and

hybrids. Defining facial and pelage characteristics from each species and hybrid type were based on published descriptions [27, 32, 31, 34, 25] and personal observations by JM and CSI. Facial landmarks used to assign sampled individuals to species are shown in Supplementary Figure S1. Phenotypes of hybrids classified as *C. aurita* hybrids suggest that these individuals possess ancestry from *C. aurita* and at least one species from the *jacchus* group [25, 34]. Previous phylogenetic analysis of mitogenomic haplotypes assigned to a subset of *C. aurita* hybrids used in our sample also support *C. aurita* x *jacchus* group ancestry in these individuals (BJT024/*C. aurita* mitogeome, BJT025/*C. jacchus* mitogenome, BJT026/*C. penicillata* mitogenome, BJT027/*C. geoffroyi* mitogenome, BJT115/*C. aurita* mitogenome) [24]. Three hybrids were not able to be classified at the species level due to ambigious phenotypes, and were therefore classified as *Callithrix* sp. x *Callithrix* sp. hybrids. The only exception was hybrid BJT070 for which previous mitogenomic phylogenetic analysis determined *C. geoffroyi* to be one of the parental species [24].

Morphometric Measurements and Analysis

Sampled adults were measured with a tape measure and digital calipers and weighed while under anesthesia, following methods described by Nagorsen and Peterson [35]. Metric data are represented by one measure of body weight (WEIGHT) taken in grams (g), and 12 linear distances. Linear distances measured in centimeters (cm) were tail length (TAIL), humeral length (HUMERUS), distance of forearm (FOREARM), body length (BODY), femur length (FEMUR), tibia length (TIBIA). Linear distances measured in millimeters (mm) were intercranial-lateral distance (IC), fronto-occipital distance (FO), widest distance between zygomatic arches (ZYG), distance between mandible angles (JAW), wrist-longest claw (HAND), and calcaneus-longest claw (FOOT). For HAND, HUMERUS, FOREARM, FEMUR, TIBIA, and FOOT measures, we measured both left and right sides on sampled individuals, and then took the bilateral average of each measurement for further analyses. Raw metric data are presented in Supplementary Table S1.

All analyses described below were carried out in R [36] and code is available in Supplementary File "Morphometricsv3_code.Rmd." To first check for normality of the data, we produced normal quantile-quantile (QQ) plots for all variables. For each variable most points fall approximately along the reference line (Supplementary Figure S2). We also inspected stem-and-leaf plots for each variable (see Results). Although some variables indicated slight deviation from normality based on these plots, the parametric statistical tests described below are fairly robust to such violation, so we left the measured traits uncorrected [37].

To test for any confounding effects from sexual dimorphism in our data, we conducted a series of parametric multivariate analysis of variance (MANOVA). We first used MANOVA to test for an interaction between sex and taxon for all 13 morphological traits, which was not statistically significant (p-value=0.9665). Grouping all 13 traits by sex indicated that these variables do not differ significantly between males and females (p-value=0.74). On the other hand, grouping all 13 traits by taxon in the MANOVA test indicated a statistically significant effect of taxon (p-value <0.0001). Based on these MANOVA tests, we do not expect there to be any confounding effects from sexual dimorphism on the thirteen morphological traits in our data set.

Following these tests, each of the 13 measurements was analyzed individually using ANOVA to test for differences between all taxa. Prior to running each ANOVA test, we checked for homogeneity of variances by Levene's test for each variable among taxa. As not all traits showed homogeneity of variance (see Results), we conducted one-way Welch's ANOVAs, which were followed up by Games-Howell post-hoc tests to perform multiple pairwise comparisons between groups. The Games-Howell test was carried out with the Rstatix [38] R package and p-values were adjusted for multiple comparisons using the Tukey method. *Callithrix* sp. x *Callithrix* sp. hybrids were not considered in these analyses due to low sample number.

For *C. jacchus* x *C. penicillata*, *C. penicillata* x *C. geoffroyi*, and *C. aurita* hybrids, we compared hybrids and parental species to determine if any traits showed evidence of heterosis, dysgenesis, or transgressive segregation. For *C. aurita* hybrids, all possible combinations of *C. aurita* and *jacchus* group species from our samples were used as putative parental species as it was not possible to determine the exact parental species of *C. aurita* hybrids. Other hybrid types were excluded from these tests due to relatively small sample numbers. First, we calculated the mid-point values (MPVs) for each possible parental pair of species for all 13 traits. We then compared trait means of each hybrid group against their respective MPVs using one-sample t-tests. Mean hybrid trait values that fell in between parental trait means and were not statistically significantly different from the MPVs were considered intermediate. Mean hybrid trait values that were significantly larger than the MPVs were considered heterotic. Mean hybrid trait values significantly smaller than the MPVs were considered dysgenic. Following this, Welch's two sample t-tests, which account for unbalanced size and lack of variance homogeneity among samples, were conducted between hybrids and each parental species. A trait was considered transgressive if the hybrid mean was larger than both parental means, and all hybrid-parental species Welch's t-tests were statistically significant.

A principal components analysis (PCA) was also performed on the data in order to visualize differences among the pure species and hybrids. This technique reduces the dimensionality of a data set producing a smaller number of uncorrelated variables that nonetheless retain all of the original size and shape information. Separate PCAs were conducted for *C. jacchus* x *C. penicillata*, *C. penicillata* x *C. geoffroyi*, and *C. aurita* hybrids. For *C. aurita* hybrids, as described above, all possible combinations of *C. aurita* and *jacchus* group species from our samples were used as putative parental species.

Genetic Distance between *Callithrix* Species

To determine mean pairwise genetic distances between *C. aurita*, *C. jacchus*, *C. penicillata*, and *C. geoffroyi*, we used previously published mitogenomic sequences [23], which included a subset of marmosets used in this current study. Samples and mitogenomic Genbank accession numbers are listed in Supplementary Table S2. Mitogenomic haplotypes were grouped by species and mean genetic distances between these groups were calculated with MEGA11 [39, 40]. We used the "Compute Between Group Mean Distance" option with default settings of the Maximum Composite Likelihood model, transitions and transversions substitutions included, uniform rates among sites, same (homogeneous) patterns among lineages, and pairwise deletion as gaps/missing data treatment.

Results

Descriptions of *Callithrix* Phenotypes

Callithrix Species Phenotypes

Examples of the *C. aurita* phenotype are shown in Figure 2A. The frontal portion of the facial vertex of *C. aurita* is beige to orange and the proximal region of the head is black. The facial menton has yellowish to orange pelage, while the facial orbital region contains a mix of yellowish and peachy pelage. The *C. aurita* ear tuft frames the facial region but the tuft hair is not as full or dense in volume as that of *C. jacchus*; the ear tufts may be black, yellow, or orange, or a mix of black with yellow tips at tuft ends. The pelage of the *C. aurita* facial lateral sides is black. The forehead, nasal, and infraorbital regions have beige to light orange pelage. Pelage on the back does not form a pattern of obvious striae, but proximally there is a mixture of orange banded patches (the orange is more intense than that of *C. jacchus* and *C. penicillata*) among black pelage. The orange coloration of the back is less intense moving proximally to distally, and becomes predominately black towards the tail base. The proximal region of the neck has black hair, but the distal region has pelage that follows the pattern described for the back. The belly region has black pelage with some slightly orange tips at the distal part of the hairs. The proximal regions of the arms and legs have black pelage with some with orange tips. The distal base of the arm has also black hair with orange tips that is more evident than in the distal part of the legs. The tail pelage has a black, grey, and orange striated pattern.

The *C. geoffroyi* phenotype is shown in Figure 2B. The frontal vertex of *C. geoffroyi* is fully white while the proximal portion of the head is black. The orbital region is peachy, but the forehead and most of the face around the orbital, nasal, and infraorbital regions is also white. The pelage of the menton region can be white or beige combined with darker hairs. The *C. geoffroyi* ear tuft pelage is very dense as in *C. jacchus*, and similar in volume, but the ear tuft hair is black. Tuft hairs closer to the top of head are shorter and tuft hairs closer to neck are longer. The neck pelage is black, and the back region has striations which can be either black and orange or black and grey. Portions of orange coloration in the pelage of the back are obvious and prominent. The proximal portions of the arms and legs are black and can be speckled with a whitish-grey coloration with overall darker coloring on the outer parts in the arms and legs. Tail pelage has a black, grey and orange striated pattern.

The *C. jacchus* phenotype is shown in Figure 2C. *Callithrix jacchus* pelage of the front half of the vertex is dominated by grey tips of hair, but can also have beige or brown tones. The back portion of the vertex is brown with tips of grey hair. The menton region pelage is grey. The facial orbital region is more peachy and buff colored than in *C. penicillata*. The *C. jacchus* tufts are periauricular, white and the hair is highly voluminous. Tips of the *C. jacchus* tuft hairs may have some black tones. The pelage on the lateral sides of face ranges from dark brown to a little orange with some hairs that may have greyish tips. A white 'star' is present and prominent on the forehead of *C. jacchus*. The upper neck region has dark brown coloration, while the lower neck region transitions towards aguti coloration. Striations present with black and whitish-grey top pelage coat and an orange colored

pelage undercoat define the back region. The arms have black to dark brown pelage and tips of pelage hairs are grey to light orange or orange. The legs follow striation pattern of the back region. Tail pelage has black, grey and orange striated pattern.

The *C. penicillata* phenotype is shown in Figure 2D. The front half of the vertex pelage is dark brown to black and the back vertex pelage is dark brown to black. The pelage of menton region is whitish-grey, while the facial orbital region pelage is creme-buffy colored. The ear tuft is preauricular and its region has thin, downward facing, relatively long black pelage. There is a prominent white 'star' present on the *C. penicillata* forehead and the pelage on the lateral sides of face is whitish-grey to dark brown. The upper and lower neck pelage has dark brown and black coloration, with occasional presence of specks of whitish-grey. Striations on the back combine a whitish-grey/black pelage topcoat with an orange pelage undercoat. Light orange to orange and black pelage is present in the central belly region of *C. penicillata*. The proximal region of the arms is predominantly whitish-grey, and the proximal region of the legs follows the striation pattern of back region. Tail pelage has black and whitish-grey striations.

Callithrix Hybrid Phenotypes

Examples of anthropogenic *C. jacchus* x *C. penicillata* hybrid phenotypes from southeastern Brazil are shown in Figure 3A. The front portion of the vertex pelage of *C. jacchus* x *C. penicillata* hybrids is composed of grey and black hair of varying intensities, while the back half of the vertex coloration may range from black to greyish and/or orange pelage. The menton region pelage is grey. Pelage of the orbital region is variable shades of orange, and may even be pink. The ear tuft pelage of *C. jacchus* x *C. penicillata* hybrids is usually less voluminous than in *C. jacchus* but more so than in *C. penicillata*. Hybrid ear-tuft coloration ranges from black with grey tips to grey with some black hair. These hybrids have a white 'star' present on the forehead, as also possessed by parental *C. jacchus* and *C. penicillata*, but the hybrid star mark varies in size. The lateral sides of the face of hybrids have pelage of greyish coloration with some black and orange hairs. Coloration of neck pelage may be black,grey, and/or orange. Hybrid back pelage has striations interspersed with orange, black, and grey coloration. The striation patterns may not be as uniform as in parental species. The intensity of orange back coloration varies among hybrid individuals. The belly pelage varies in intensity from black to orange, but these two colors are striated. Pelage on the proximal region of the legs follows the pattern of the back region. The proximal regions of the arms have black to dark brown fur with grey tips. The tail pelage has black, grey and orange striated pattern, varying in color intensity.

Examples of anthropogenic *C. geoffroyi* x *C. penicillata* hybrid phenotypes from Viçosa, Minas Gerais are shown in Figure 3B. For these hybrids, the front and back halves of the vertex pelage follows pattern of the lateral sides of the face, which varies in intensity from white to grey. Pelage of the upper neck of the hybrids varies from white to dark grey. In the lower neck part, the hair can be black and may have grey tips. In the facial menton region of hybrids, pelage follows the pattern of lateral sides of the face. In the facial orbital region, hybrids have pelage that is slightly orange or peachy. The hybrid ear tuft pelage color is black but the volume

of tufts varies between that of the parental species. The white forehead mark of *C. penicillata* is present in these hybrids but varies in intensity between individual hybrids. The pelage of the back region possesses patterns of black, grey and orange streaks, as seen in the parental species. Black hairs are found in the central part of the belly, but the hairs are intense orange in the outer parts of the belly. The proximal portion of the legs follows the pelage pattern of the back, and the proximal portion of the arms has black hairs with some grey tips. The tail pelage shows a black, grey and orange striated pattern.

Examples of anthropogenic *C. aurita* x *Callithrix* sp. hybrid phenotypes are shown in Figure 3C-E. *Callithrix aurita* x *Callithrix* sp. hybrids have a facial front vertex with black and grey hairs that have orange tips. In the back half of the vertex, the pelage coloration contains black hair with grey tips, with variation in the intensity of the grey. The vertex of some hybrid individuals will have patches of whitish grey and grey mixed in with the darker black pelage hairs. This pattern also occurs in the neck region. The menton region pelage is whitish-grey, and the orbital region pelage may be peachy as in *C. jacchus* and *C. penicillata*, or yellowish like *C. aurita*. Hybrid ear tuft hair volume may be sparse like *C. aurita* and *C. penicillata* or very dense like *C. jacchus*, varying in the amount of black, grey, and orange hair at the hair tips. Some hybrids possess a white star on the forehead. Others will have a *C. aurita*-like pattern where the forehead, orbital, nasal, infraorbital, and mentonian facial regions have beige to light orange hairs. The lateral sides of the face have black to dark brown hair that may or may not have grey tips.

Unlike *C. aurita*, *C. aurita* x *Callithrix* sp. hybrids show back striation patterns that are similar to that of *C. penicillata* and *C. jacchus*. The striations may contain a mixture of black, grey and orange patterns or black and whitish-grey streaks. In *C. aurita* x *Callithrix* sp. hybrids, the orange color of back pelage tends to be more intense than in *C. aurita*, and greys of the back pelage are more yellowish or orange instead of whitish than in *C. penicillata* and *C. jacchus*. Belly coloration is highly variable between hybrids. The proximal region of legs follows the pattern of the back. The proximal portion of the arm has black fur with grey to orange tips. The hybrid tail pelage has a black and grey striated pattern and there may be orange coloration at hair tips. The hands of these hybrids tend to have an orange or yellow tone, similar to *C. aurita*.

Example of a *Callithrix* sp. x *Callithrix* sp. hybrid phenotype from Santa Teresa, Espírito Santo is shown in Figure 3F. For this hybrid, the front of vertex pelage is yellowish with a mix of grey and black speckles, and the back of the vertex pelage is black with greyish speckles. Pelage of the facial menton region is dark. The facial orbital region pelage is black towards the eyes and peachy on the outer regions. A white forehead star is present in these hybrids. The ear tuft pelage is very dense as in *C. geoffroyi*. Hybrid ear tufts are black, and hairs closer to top of head are shorter and hairs closer to neck are longer. The upper neck region has black hair, while the lower neck portion has greyish tips. Pelage in the back has striations that are black\orange and black\grey. The orange coloration is very obvious and prominent in the hairs of the back pelage. The belly pelage contains striations of black and grey. The proximal leg portion is black and the proximal arm region has whitish-grey hairs. The individual pictured in Figure 3F likely possesses ancestry from *C.*

penicillata or *C. jacchus* given the forehead star, as well as previously confirmed *C. geoffroyi* ancestry. However, this phenotype is distinct from that described for *C. penicillata* x *C. geoffroyi* hybrids described above.

Callithrix Morphometric Traits

The means, standard deviations, and sample number for each *Callithrix* morphological trait are listed in Tables 2-5 and box-plots for each trait are shown in Figure 4. For all cases, traits are grouped by *Callithrix* taxon. Box plots (Figure 4) show that trait median values for *Callithrix aurita* tends to be the largest across all taxa. Median trait values for *C. geoffroyi* show a similar pattern within the *jacchus* group, whereas *C. jacchus* and *C. penicillata* tend to be the smallest among all taxa. Among the hybrid groups, the *C. aurita* hybrids tended to have the largest median values for all measured traits. On the other hand, *C. penicillata* x *C. jacchus* hybrids showed the smallest median values for most traits.

Quantitative differences among the purebred taxa and their hybrids are significant (parametric MANOVA $F(91, 910) = 2.7957$, $p<0.01$). Prior to conducting univariate ANOVA tests, we generated normality QQ plots for each respective trait (Figure S2). Levene's test indicated that the BODY, IC, FO, FOREARM, FEMUR, TIBIA, and FOOT traits had homogeneity of variance with p-value >0.05. All other traits produced significant p-values (<0.05) for Levene's test. Univariate Welch's ANOVA tests (Supplementary Table 3) indicate significant differences for mean values of all traits among all taxa. Games-Howell post-hoc tests are shown in Supplementary Table 4.

Among species, we consistently see significant differences between *C. aurita* and *C. jacchus* and *C. penicillata* across most mean trait values. Only in the HAND trait did post-hoc tests fail to find significant differences in pairwise comparisons among species. Among *jacchus* group species, *C. geoffroyi* was significantly different for a larger number of traits when compared with *C. jacchus* than with *C. penicillata*. The FEMUR, TIBIA, and HUMERUS means of *C. geoffroyi* was significantly different from that of both *C. jacchus* and *C. penicillata*. There were no significant differences between *C. jacchus* and *C. penicillata* trait means.

For hybrids and their parental species, *C. aurita* hybrids were not significantly different from *C. aurita* nor *C. geoffroyi* for any trait means based on post-hoc tests. There was a significant post-hoc difference in WEIGHT and FEMUR means between *C. aurita* hybrids and *C. jacchus*. A post-hoc difference in WEIGHT means was also significant between *C. aurita* hybrids and *C. penicillata* based on the parametric post-hoc test. For *C. geoffroyi* x *C. penicillata* hybrids and *C. geoffroyi*, there were no significant post-hoc differences for any traits. On the other hand, *C. geoffroyi* x *C. penicillata* hybrids were significantly different from *C. penicillata* for almost half of measured traits. There were no significant differences between *C. jacchus* x *C. penicillata* hybrids and either of the parental species in post-host testing.

Results of hybrid-parental species comparisons for heterosis, dysgenesis, intermediacy, and transgressive segregation are shown in Tables 3-5. For *C. jacchus* x *C. penicillata* hybrids, most traits were larger than the parental species, though only a subset of these traits was significantly larger. Heterosis among these hybrids is

shown in the TAIL, BODY, and IC traits, and no traits displayed evidence for dysgenesis. FOOT and WEIGHT traits were intermediate between *C. jacchus* x *C. penicillata* hybrids and their parental species. For *C. penicillata* x *C. geoffroyi* hybrids, we found evidence for heterosis in the ZYG, TAIL, TIBIA, and FEMUR traits, while FO and JAW showed evidence of dysgenesis. The BODY, WEIGHT, and IC traits in *C. penicillata* x *C. geoffroyi* hybrid traits were intermediate, and none were transgressive. Among *C. aurita* x *Callithrix* sp. hybrids, we found evidence for transgressive segregation in the hand when parental species combinations were *C. aurita*/*C. penicillata* and *C. aurita*/*C. jacchus*. About half of traits for *C. aurita* x *Callithrix* sp. hybrids were intermediate between all parental combinations of *C. aurita* and *jacchus* group species. *Callithrix aurita* x *Callithrix* sp. hybrids jaw means were larger than those of the parental species, but there was no statistical support for heterosis or transgression.

Component scores for hybrid individuals and their parental species are plotted in Figure 5A-C. For *C. jacchus* x *C. penicillata* hybrids and parental species, Figure 5A as well as the positive loadings of PC1 (38.12% of variance) indicated a high degree of overlap between hybrids and parental species for overall size. PCA eigenvalues for this analysis are shown Supplementary in Table S5. Figure 5A indicates that hybrids on average occupy an intermediate space shape between their parental species, but hybrid variation magnitude exceeds that of the parental species (Supplementary Table S6). Other PCs beyond PC1 of the *C. jacchus* x *C. penicillata* hybrids and parental species PCA combined positive and negative values indicating that they portray aspects of shape (Supplementary Table S6).

The PCA for *C. penicillata*, *C. geoffroyi*, and their hybrids (Figure 5B) shows a strong separation between the two parental species along PC1 (42.90%), with larger *C. geoffroyi* towards the left and smaller *C. penicillata* towards the right. PCA eigenvalues for this analysis are shown in Supplementary Table S7. Hybrids fall in between the two parental species along PC1 and PC2, indicating that the magnitude of variation in the sampled hybrids does not exceed that of parental species (Supplementary Table S8). The negative loadings of PC1 of this PCA may portray aspect of overall size. PC2 shows positive and negative values which may portray shape aspects among *C. penicillata*, *C. geoffroyi*, and their hybrids (Supplementary Table S8).

The PCA plot of the four study species and *C. aurita* x *Callithrix* sp. hybrids (Figure 5C) shows more overlap between the three *jacchus* group species to the exclusion of *C. aurita* along PC1 (42.90% of variance). *Callithrix aurita* is leftmost, followed by *C. geoffroyi*, and then a large area of overlap between *C. jacchus* and *C. penicillata*. PC1 in Figure 5C seems to be influenced by both size and shape of the marmosets. The hybrids cluster closest to *C. aurita* toward the left side. However, both *C. aurita* and the hybrids show a great deal of variability along PC1 and PC2. PCA eigenvalues for this analysis are shown in Supplementary Table S9. All negative loading on PC1 indicate that this may be an overall size component (Supplementary Table S10). PC2 (17.76% of variability) seems heavily influenced by jaw, FO, and hand (Supplementary Table S10). The magnitude of *Callithrix aurita* hybrid variation magnitude exceeds that of the all parental species (Figure 5C).

Callithrix Species Mitogenomic Genetic Distances

Mean pairwise mitogenomic genetic distance between *C. jacchus*, *C. penicillata*, *C. geoffroyi*, and *C. aurita* are listed in Table 6. These measures show that *C. jacchus* and *C. penicillata* possessed the smallest mean distance out of all pairwise comparisons. Then *C. geoffroyi* had the same genetic distance from both *C. jacchus* and *C. penicillata*. Finally, *C. aurita* was the most genetically removed from all three other species.

Discussion

Pelage Variation in *Callithrix* Species and Hybrids

Several hypothesis have been put forth to explain the overall phenotypic variation in primate coloration which include protection, communication, character displacement, physiology, and sexual selection [41, 42, 43], some of which may explain *Callithrix* species coloration. For example, Gloger's rule predicts that endothermic animals will be darker in wetter, more humid locations [41, 44], and evidence has been found for this pattern in primates [45]. *Callithrix aurita* has the darkest overall pelage coloration of all *Callithrix* species, and occurs in some of the highest average rainfall regions of the *Callithrix* natural geographical range [23, 46]. On the other hand, *C. jacchus* and *C. penicillata*, which inhabit the semi-arid regions of Brazil known as Caatinga and Cerrado [23, 46], do indeed show lighter pelage than other *Callithrix* species. *Callithrix jacchus* also naturally occupies a narrow strip of the humid Atlantic Forest, and Caatinga *C. jacchus* populations have a lighter topcoat than Atlantic Forest populations (pers. obs., JM). It is plausible that these differences in marmoset pelage coloration can be partially explained by Gloger's rule. Under character displacement, the intricacy of pelage coloration is used by individuals to distinguish conspecifics from heterospecifics to reduce the probability of hybridization [41]. Although marmosets naturally have separate geographical ranges, there are cases of natural contact zones between species [24, 25], and the distinct pelage coloration in these zones could be used by individuals for mating choices. For facial color patterns, evidence was found for Neotropical primates living in smaller groups having more complex facial pattern than Neotropical primates living in larger groups [42]. The same study also found that as species' ranges go from semi-arid regions like the Caatinga and Cerrado to forested environment of the Amazon, primates have darker regions around the eyes, lighter nose and mouth, and shorter hairs around the face [42]. The marmoset species which inhabit the Caatinga and Cerrado, *C. jacchus* and *C. penicillata* do indeed show lighter pelage around the eyes and darker tones around the mouth and nose.

Callithrix hybrids pelage patterns and coloration incorporate parental phenotypes into novel combinations, which extends hybrid phenotypic pelage variability beyond that of what is normally seen in parental species [23, 25, 31, 32]. One study recently suggested that multigenerational marmoset hybrids experience a "greying out" of parental pelage coloration as hybridization goes on over time and that parental characteristics are only distinguishable in early generation hybrids [47]. However, data on pelage phenotypes presented in this study and previously published studies do not sustain this prediction. For example, in several late-generation natural and anthropogenic hybrid zones between *jacchus* group species, parental phenotype

and genotype combinations, respectively, are uncoupled within hybrid populations and reshuffled into new combinations amongst hybrid individuals [23, 25, 31, 32]. Parental pelage characteristics and coloration are still observable in anthropogenic marmoset hybrid zones that have existed for over 30-40 years (that is about 45-60 marmoset generations assuming a marmoset generation time of 1.5 year), and that do not receive natural gene flow from parental species [31, 32].

The greyish marmoset hybrids exemplified by Vital et al. [47], are similar in pelage phenotype to the *C. aurita* x *jacchus* group hybrids we present in this study and also discussed in [25]. These marmosets hybrids are greyer in appearance than *jacchus* group hybrids, but also retain pelage characteristics indicative of ancestry from both *aurita* and *jacchus* group marmoset species. Genomic data on global admixture levels for the *C. aurita* x *Callithrix* sp. hybrids in our study (unpublished data, Malukiewicz) suggest that these are likely late generation hybrids, which goes against any progressive greying-out of pelage hypothesis in such hybrids.

Morphometric Variation in *Callithrix* Species

Marmoset cranial shape and musculature, dentition, in addition to digestive features [48, 49, 50, 51], support *Callithrix* exudivory by allowing marmosets to gouge and scrape hard plant surfaces to access and digest natural exudate sources made of hard to digest oligosaccharides [48, 52, 53, 54, 51, 50, 49, 55, 56, 57, 58, 59, 60]. However, interspecific differences in marmoset cranial shape and dentition *Callithrix* species are linked to intersepcific differences in exudivory specializaiton [52, 61, 62, 63], with *C. jacchus* and *C. penicillata* representing the extreme of marmoset exudivory specialization and *C. aurita* being the least specialized [64]. *Callithrix penicillata* and *C. jacchus* have compressed braincases and more protruding dentition in comparison to *Callithrix aurita* and *C. flaviceps* [52]. Specifically in *C. jacchus*, the cranial musculoskeletal configuration allows for the use of extreme wide jaw gapes to gouge tree holes with the anterior dentition. In our results for cranial traits (IC, FO, ZYG, and JAW) [54, 56], we saw significant pairwise differences between *C. aurita* - *C. jacchus* and *C. aurita* - *C. penicillata* comparisons while all pairwise comparisons between *C. jacchus* and *C. penicillata* were not significant. Other studies have reported either no significant differences or a high degree of overlap in *C. jacchus* and *C. penicillata* cranial and dental traits and that these species are morphological distinct in such traits from *C. aurita* [62, 63, 52]. We attribute the differences seen in craniofacial morphology of marmoset species in our results to differences in exudivory specialisation between these species [24, 64].

Primate exudivores tend to be small in size [59], and in our study the most extreme marmoset exudivores, *C. jacchus* and *C. penicillata* were on average the smallest for all thirteen morphological traits. Then as with cranial traits, these two species were the only pair which did not possess any significant pairwise trait differences for post-cranial traits. On the other hand, *C. aurita* as the least specialized species for exudivory, tended on average to be the largest for most of the thirteen studied morphological traits. These species respectively represent the two relative extremes of exudivory in *Callithrix*, with the other marmoset species falling somewhere in between as far as exudate consumption [24]. Morphologically, *C. geoffroyi* fell in between the rest of the species included here. Other morphological studies of the

marmoset cranium show that *C. flaviceps* is most similar to *C. aurita* and *C. kuhlii* is closer to the other four *Callithrix species* [52, 63, 63, 61]. These trends also reflect level of exudivory specialization in these other species [24].

Morphometric Variation in *Callithrix* Hybrids and their Parental Species

Our results show that patterns of hybrid phenotypic variation relative to parental species is not consistent among marmoset hybrids with differing parental species ancestries. We see the least amount of MPV deviation in hybrids with the least mitogenomic genetic distance between the parental species, that being *C. jacchus* and *C. penicillata*, with five intermediate traits and two traits with heterosis. Intermediate traits of these hybrids show a mix of being closer to the trait mean of one parental species than the other. Then *C. penicillata* x *C. geoffroyi* hybrids, whose parental species possess larger mitogenomic distance show 5 traits with heterosis, one with dysgenesis, and 4 intermediate traits. In the latter set of traits, three were closer to *C. geoffroyi* means than *C. penicillata* means. A previous study of *C. penicillata* x *C. geoffroyi* hybrids in the same sampling locality as ours also found that for traits which fell within the parental species range, hybrids were closer to *C. geoffroyi* than *C. penicillata* [32]. For the *C. aurita* hybrids, WEIGHT was heterotic in *C. aurita*-*C. penicillata* contrasts, which are putative parental species pairs with a relatively high level of genetic differentiation. All other traits fell into the range of all possible putative parental species, but 5 were closest to *C. aurita* and three were closer to *C. aurita* and *C. geoffroyi*.

Underlying differences in the degree of genetic similarity between parental taxa of hybrids are important factors in determining patterns of phenotypic variation in hybrids [8, 17, 18, 20]. Mitogenomic data for a subset of our sampled marmosets show that *C. jacchus* and *C. penicillata* are most similar to each other, and *C. geoffroyi* is closer to these two species than to *C. aurita*. Also as previously discussed, *C. jacchus* and *C. penicillata* share similar adaptations to gumnivory which are not seen to the same degree as *C. geoffroyi* and especially not in *C. aurita*. Evidence from experimental hybridization also supports these same patterns of genetic similarities and differences between marmoset species [24].

Large differences in gene frequencies between parental populations are expected to contribute to the occurrence of heterosis and dygenesis in hybrids [18]. Therefore, dysgenesis should occur in more distantly related and adaptively distinct species [8]. Due to their genetic closeness and adaptive similarities, there is likely less breakdown of co-adaptive gene complexes between *C. jacchus* and *C. penicillata* than between other pairings of *Callithrix* parental species in our sample. We also probably see less heterosis in *C. jacchus* x *C. penicillata* hybrids than in other hybrid types in our sample as there may be a lesser amount of differentially fixed alleles between *C. jacchus* and *C. penicillata* than between other marmoset species. Due to the relatively less genetic and adaptive similarity between *C. penicillata* and *C. geoffroyi* than between *C. jacchus* and *C. penicillata*, our results suggests some breakdown of co-adaptive gene complexes, and higher number of different alleles that have been fixed between the former than latter pair of parental species.

Transgressive hybrids are those which show extreme phenotypes that exceed the parental species phenotypic range [17]. Transgression in hybrids is expected to increase with greater genetic distance between interbreeding parental species due

to complementary gene action or epistasis [17]. We observed transgression in the HAND trait of *C. aurita* hybrids between *C. aurita*-*C. jacchus* and *C. aurita*-*C. penicillata* contrasts, which represent the most genetically distant pairing of parental species in our sample. PCA plots of *C. jacchus* and *C. penicillata* show that most hybrids fall within the range of parental species phenotypic variation, but a few extreme hybrid individuals outside of the parental range represent transgressive individuals. Interestingly, we did not see indication of trangressive hybrids in PCA plots of *C. geoffroyi* x *C. penicillata* hybrids and parental species, while Fuzessy et al. [32] did. This difference maybe due to a larger number of hybrids sampled by Fuzessy et al (N=40) than in this study (N=18). For *aurita* x *jacchus* group hybrids, most of these individuals are transgressive that fall outside the phenotyptic range of all four parental species. Thus, transgressive hybridization in marmosets, when considering morphometric shape and size in terms of genetic relatedness between parental species, follows theoretical expectations.

Implications of Understanding Marmoset Hybrid Pelage and Morphometric Diversity

As pointed out by Ackermann [8], a lingering question about the evolutionary importance of hybrid phenotypic expression is "to what extent might differences in the expression of hybrid traits exist due to degree of temporal divergence?" Our results based on *Callithrix* show that indeed expression of morphometric traits differs in hybrids resulting from interbreeding between different combinations of closely-related parental species that differ in genetic distance. Temporal divergence between parental marmoset species included in this study tracks positively with their level of genetic distance [24]. Further, experimental hybrid crosses showed that *C. jacchus* and *C. penicillata* hybridize relatively more easily than other *Callithrix* species pairing, and their hybrid progeny also show relatively less physical abnormalities (see [25]). Thus, our empirical data and past experimental data suggest that less developmental disturbances can be expected in hybrids of species that have diverged relatively more recently. Given the various anthropogenic hybrids found across southeastern Brazil, *Callithrix* marmosets represent a system where this question can be explored more directly for phenotypes related to anatomy and beyond experimental setting. Further tests of this question should combine phylogenetic, genomic, and phenotypic data from sampled hybrids and their parental species and consider underlying genetic architecture of a given trait and generational age of hybrids. Combining these factors will provide a fuller understanding of hybrid phenotypic expression, and provide insight into how natural animal populations may evolve as anthropogenic hybridization continues to increase.

For marmosets themselves, establishing a firm understanding of phenotypic differences and variability in both *Callithrix* species and hybrids is important for both evolutionary, conservation, and applied reasons. Anthropogenic marmoset hybrids and exotic marmosets regularly fill up governmental and zoological captive facilities in Brazil and marmoset species such as *C. jacchus* are usually kept in biomedical facilities outside of Brazil. Pelage colors and patterns that are easily observable and distinguishable are usually the first key characteristics to classify a marmoset individual as either a hybrid or non-hybrid as well as the likely ancestry of that species. Anthropogenic hybrids pose ecological and conservation challenges, particularly in southeastern Brazil, but natural marmoset hybrids are also found along

the entire geographical *Callithrix* range. Thus proper identification of marmoset hybrid and ancestral status is fundamental in execution of any marmoset conservation and population management plans in and out of captivity. Our suggestions to this end include adopting and developing quantitative approaches and tools towards identification and taxonomic classification of marmosets, as most approaches still depend on subjective, qualitative descriptions description which are subject user error. A future direction could also involve the development of a machine-learning phone app to help biological and clinical workers easily identify marmosets. Ideally, phenotypic data should be combined with mitochondrial and nuclear genome data in identification and classification of marmosets, as phenotypic data is not fully reliable to this end as cryptic hybridization does occur in marmosets [25, 65].

Declarations

Ethics approval and consent to participate

Animals were sampled under the approval of the Arizona State University Institutional Animal Care and Use Committee Animals (ASU IACUC, protocols #11-1150R, 15-144R), Brazilian Environmental Ministry (SISBIO protocols #47964-2 and #28075-2), and a CPRJ internal review. All methods were carried out in accordance with relevant guidelines and regulations. All methods are reported in accordance with ARRIVE guidelines (https://arriveguidelines.org) for the reporting of animal experiments.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests

The authors declare that they have no competing interests.

Author's contributions

JM, KW, IOS, and RRA formulated the idea for the study. KW and RRA formulated analytical aspects of this work. KW conducted data analyses of this work. JM collected samples, obtained funding, conducted analyses, and wrote the original manuscript. ILCA aided in field and data collection. NHAC provided field assistance and logistical support. FTD, LSF, HRF, LM, VSP, and BP provided veterinary oversight and logistical support for field collections at PET. CVM assisted in phenotypic identification of hybrids, gave field assistance, veterinary oversight, and aided in data collection. CSI gave access and provided logistical and veterinary support to collect samples from animals kept at Guarulhos Zoo. AP gave access to collect samples at Centro de Primatologia do Rio de Janeiro. SBM provided logistical support and veterinary assistance to collect samples from animals kept at the Centro de Primatologia do Rio de Janeiro. TZ, MSN and JLS provided access, logistical support, and veterinary assistance at DEPAVE. PAN and LCMP gave access and provided logistical support to collect samples from animals kept at CEMAFAUNA. DLS and MOMS provided assistance in field and data collections. JR contributed towards writing the original manuscript. AAQ provided veterinary oversight and collection assistance at CEMAFAUNA. VB and IOS provided field and logistical assistance. All authors read and approved the final manuscript.

Acknowledgements

We would like to thank the Callitrichid Research Center, the Golden Lion Tamarin Association, CEMAFAUNA, Rio de Janeiro Primatology Center, the Guarulhos Municipal Zoo, the Beagle Lab at UFV, and many biologists, field technicians, veterinarians, and other individuals that made this research possible.

Funding

This work was supported by a Brazilian CNPq Jovens Talentos Postdoctoral Fellowship (302044/2014–0), a Brazilian CNPq DCR grant (300264/2018–6), an American Society of Primatologists Conservation Small Grant, a Marie-Curie Individual Fellowship (AMD-793641-4) and an International Primatological Society Research Grant for JM. RRA was supported by the National Research Foundation of South Africa.

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Figures

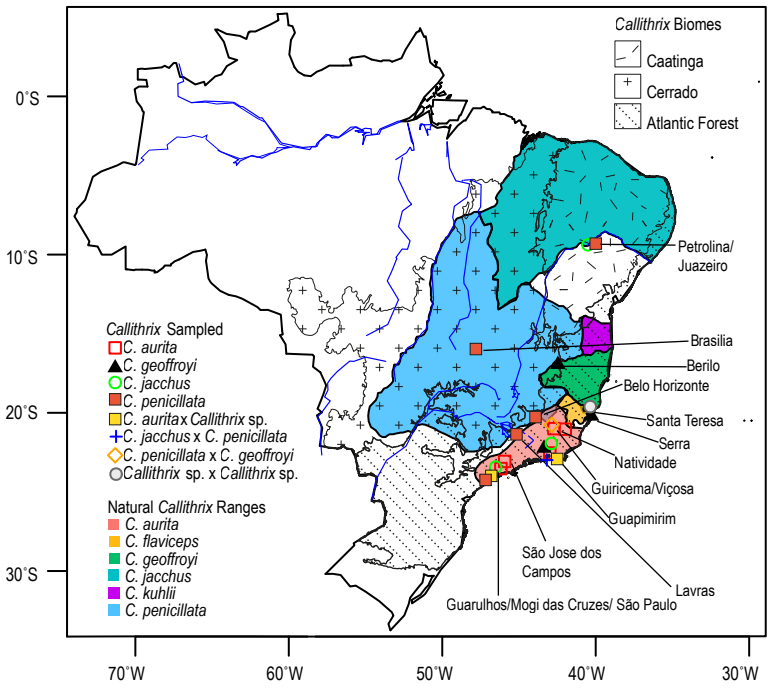

Figure 1 Marmoset sampling locations. Locations are indicated by capital letter symbols, and approximate distribution of *Callithrix* species in Brazil (2012 IUCN Red List Spatial Data; http://www.iucnredlist.org/technical-documents/spatial-data). Locations of three biomes where *Callithrix* occur naturally, the Caatinga, Cerrado, and Atlantic Forest, are also indicated.

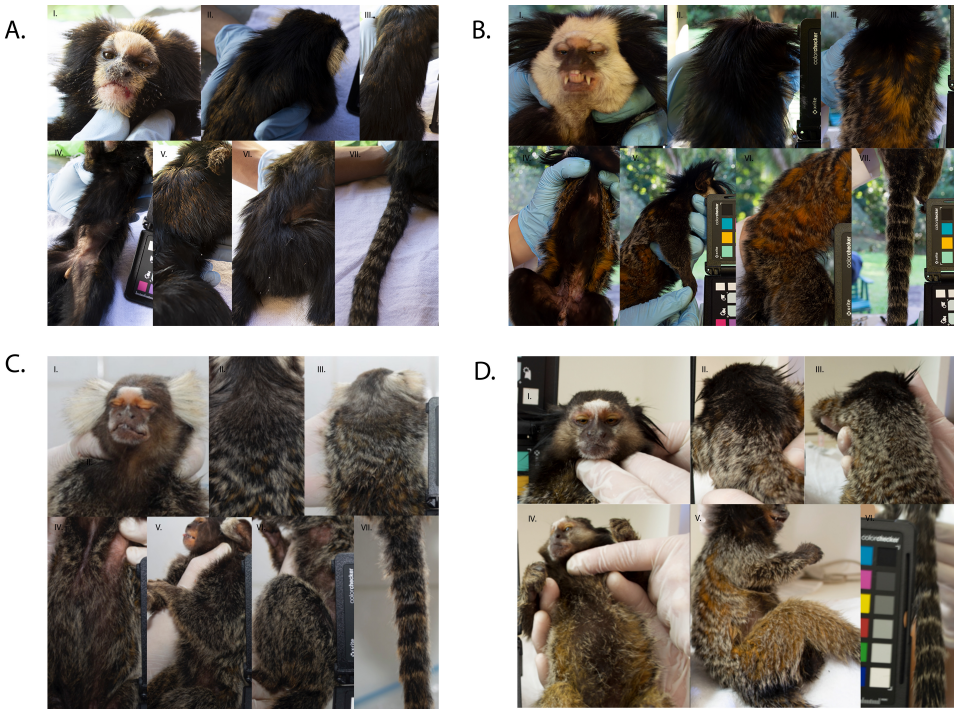

Figure 2 Phenotypes of four Callithrix species. Part A shows the *C. aurita* face and ear tufts (I), neck and upper back (II), full back (III), belly (IV), arm (V), leg (VI), and tail (VII). Part B shows the *C. geoffroyi* face and ear tufts (I), neck (II), full back (III), belly (IV), arm (V), leg (VI), and tail (VII). Part C shows the *C. jacchus* face and ear tufts (I), neck and upper back (II), full back (III), belly (IV), arm (V), leg (VI), and tail (VII). Part D shows the *C. penicillata* face and ear tufts (I), neck and upper back (II), back (III) belly (IV), arm and leg (V), and tail (VI).

Figure 3 Phenotypes of Callithrix hybrids. Part A shows examples of *C. jacchus* x *C. penicillata* hybrid face and ear tufts (I), neck and upper back (II), back (III), belly (IV), arm (V), leg (VI), tail (VII), and further facial variation (VIII-X). Part B shows examples of *C. penicillata* x *C. geoffroyi* hybrid face and ear tufts (I), neck (II), back (III), belly (IV), arm in upper right of photograph (V), leg (VI), and tail (VII). Part C shows an example of a *C. aurita* hybrid phenotype for face and ear tufts (I), neck and upper back (II), full back (III), belly (IV), arm (V), leg (VI), and tail (VII). Part D shows an example of another *C. aurita* hybrid phenotype for face and ear tufts (I), neck and upper back (II), arm (III), and belly (IV). Part E shows an example of another *C. aurita* hybrid phenotype for face and ear tufts (I), neck and upper back (II), belly (III), arm in upper portion of photograph (IV), leg in lower portion of photograph (V), and tail (VI). Part F shows an example of a *C. geoffroyi* x *Callithrix* sp. hybrid phenotype for face and ear tufts (I), neck and upper back (II), back (III), belly(IV), arm (V), and leg (VI).

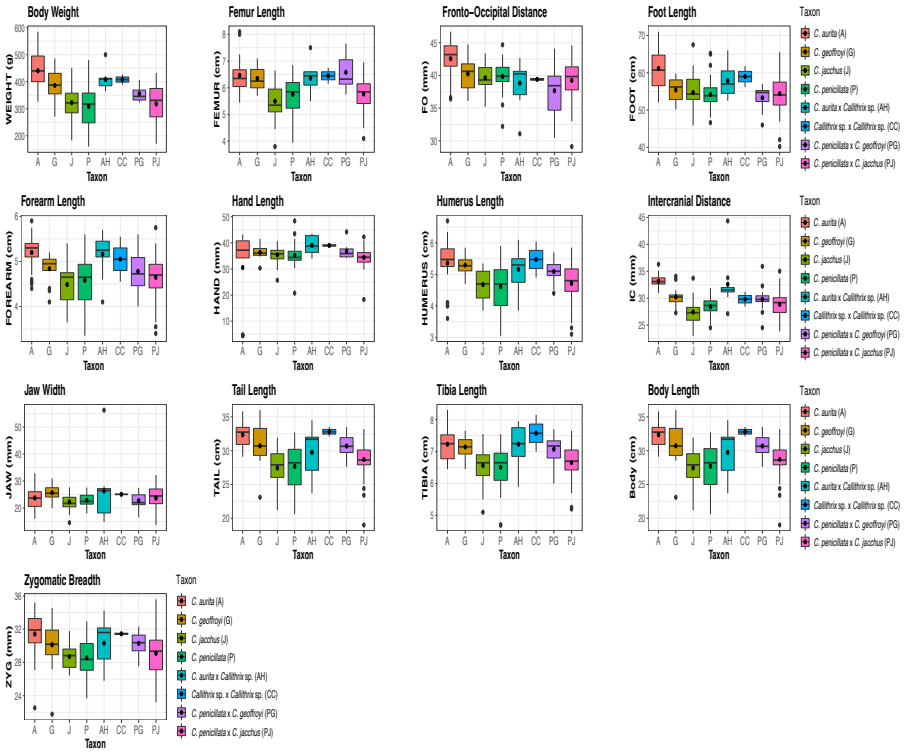

Figure 4 Stem and leaf box plots for 13 morphological traits in four Callithrix species and their hybrids. Taxon abbreviations along the x-axis in each plot are as follows: A- *C. aurita*, G-*C. geoffroyi*, J- *C. jacchus*, P- *C. penicillata*, AH- *C. aurita* x *Callithrix* sp. hybrid; CC- *Callithrix* sp. x *Callithrix* sp. hybrid; PG- *C. geoffroyi* x *C. penicillata* hybrid; PJ- *C. penicillata* x *C. jacchus* hybrid.

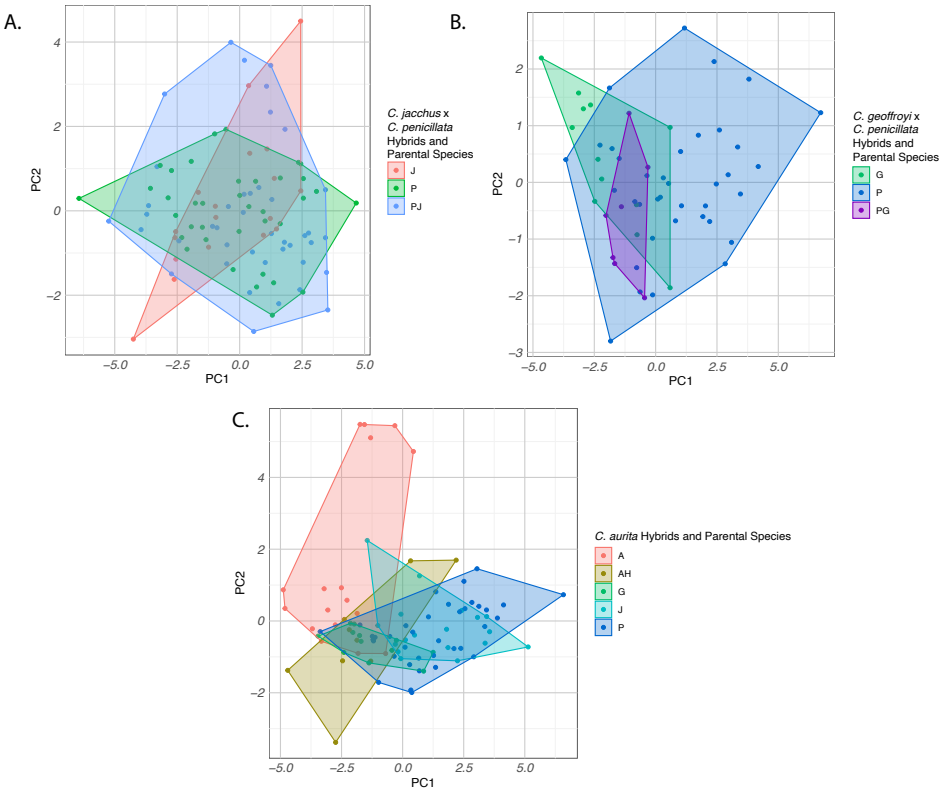

Figure 5 PCA plots for 13 morphological traits in Callithrix hybrids and their species. Bivariate plots of scores for the first two principal components/factors labelled and colored to indicate taxon affiliation. Plot A shows *C. jacchus*, *C. penicillata* and their hybrids. Plot B shows *C. penicillata*, *C. geoffroyi*, and their hybrids. Plot C shows *C. aurita*, *C. jacchus*, *C. geoffroyi*, *C. penicillata*, and their hybrids. Plot legends indicate taxon affiliation as follows: A= *C. aurita*, G = C. geoffroyi, P = *C. penicillata*, JP= *C. jacchus* x *C. penicillata* hybrids, PG=*C. penicillata* x *C. geoffroyi* hybrids, AH= *C. aurita* hybrids.

Tables

Table 1 Marmoset sample size by taxon

Taxon	N
C. aurita	27
C. aurita x *Callithrix* sp.	9
*Callithrix*sp. x *Callithrix* sp.	2
C. geoffroyi	14
C. jacchus	30
C. penicillata	55
C. penicillata x *C. geoffroyi*	18
C. penicillata x *C. jacchus*	54

Table 2 Summary of species means, standard deviations (SD), and sample numbers (N) of thirteen *Callithrix* morphological traits

	C. aurita (A)			*C. geoffroyi* (G)			*C. jacchus* (J)			*C. penicillata* (P)		
Trait	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
BODY (cm)	27	21.9	1.4	14	22.2	1.9	29	19.9	1.6	52	20.9	2.7
FEMUR (cm)	27	6.5	0.6	14	6.3	0.5	29	5.5	0.7	54	5.8	0.6
FO (mm)	27	42.6	2.9	14	40.2	2.6	24	39.7	2.2	50	39.8	2.1
FOREARM (cm)	27	5.2	0.4	14	4.8	0.3	29	4.5	0.4	54	4.6	0.5
FOOT (mm)	24	61.1	5.6	14	55.4	3.2	27	54.7	4.6	54	54.2	3.7
HAND (mm)	21	30.5	15.1	13	36.3	2.7	28	35.4	3.3	48	35.2	4.0
HUMERUS (cm)	26	5.4	0.8	14	5.3	0.3	29	4.7	0.5	54	4.6	0.7
IC (mm)	27	33.1	1.3	14	30.2	1.9	29	27.4	2.2	54	28.4	1.6
JAW (mm)	23	23.7	3.9	14	25.7	3.1	29	22.2	2.9	52	22.9	2.3
TAIL (cm)	26	32.3	1.7	13	30.7	3.2	24	27.4	2.9	51	27.7	3.2
TIBIA (cm)	27	7.2	0.5	14	7.1	0.3	29	6.6	0.6	54	6.5	0.6
WEIGHT (g)	25	440.6	66.8	14	386.2	63.0	30	322.6	65.2	54	308.4	68.1
ZYG (mm)	23	31.4	2.6	14	30.1	3.3	29	28.7	1.5	51	28.6	2.1

Table 3 Summary of species means, standard deviations (SD), sample numbers (N), mean mid-parental values (MPV) for thirteen morphological traits in *Callithrix aurita* x *Callithrix* sp. hybrids (AH). MPV_A_J= MPV between *C. aurita* and *C. jacchus*, MPV_A_P= MPV between *C. aurita* and *C. penicillata*, MPV_A_G= MPV between *C. aurita* and *C. geoffroyi*, M_AJ =p-values from t-tests from hybrids to MPV_A_J, M_AG=-p-values from t-tests from hybrids to MPV_A_G, M_AP= p-values from t-tests from hybrids to MPV_A_JP. AH_A= p-value of t-test between hybrids and *C. aurita*, AH_J= p-value of t-test between hybrids and *C. jacchus*, AH_G= p-value of t-test between hybrids and *C. geoffroyi*, AH_P= p-value of t-test between AH hybrids and *C. penicillata*. Significant p-values are indicated as "*" for p-value<0.05, as "**" for p-value<0.01, and as "***" for p-value<0.001.

	C. aurita x *Callithrix* sp. (AH)												
Trait	N	Mean	SD	MPV_A_J	MPV_A_P	MPV_A_G	M_AJ	M_AG	M_AP	AH_A	AH_J	AH_G	AH_P
BODY (cm)	9	21.4	1.9	20.9	21.4	22.0	0.488	0.307	0.946	0.433	0.056	0.327	0.534
Femur (cm)	9	6.4	0.6	6.0	6.1	6.4	0.083	0.786	0.231	0.625	**	0.980	*
FO (mm)	9	38.8	3.6	41.1	41.2	41.4	0.094	0.064	0.083	*	0.538	0.326	0.433
FOREARM (cm)	9	5.2	0.5	4.8	4.9	5.0	0.103	0.434	0.156	0.847	0.004	0.123	**
FOOT (mm)	9	57.9	4.5	57.9	57.7	58.3	0.971	0.796	0.908	0.100	0.902	0.177	*
HAND (mm)	9	39.0	3.7	33.0	32.9	33.4	***	0.908	***	*	*	0.082	*
HUMERUS (cm)	9	5.2	0.8	5.0	5.0	5.32	0.590	0.524	0.505	0.510	0.100	0.620	0.067
IC (mm)	9	32.6	4.8	30.3	30.8	31.7	0.185	0.584	0.287	0.737	**	0.187	0.030
JAW (mm)	9	26.3	12.4	23.0	23.3	24.7	0.445	0.708	0.488	0.557	0.357	0.882	0.432
TAIL (cm)	9	29.7	3.6	29.9	30.0	31.5	0.910	0.176	0.837	0.066	0.110	0.527	0.134
TIBIA (cm)	9	7.2	0.7	6.9	6.9	7.2	0.163	0.847	0.136	1.000	*	0.719	*
WEIGHT (g)	9	408.1	39.6	381.6	374.5	413.38	0.079	0.700	*	0.096	***	0.318	***
ZYG (mm)	9	30.3	3.0	30.1	30.0	30.8	0.808	0.665	0.756	0.355	0.157	0.886	0.129

Table 4 Summary of species means, standard deviations (SD), sample numbers (N), mean mid-parental values (MPV) for thirteen morphological traits in *Callithrix penicillata* x *Callithrix jacchus* hybrids. M=p-values from t-tests from hybrid to MPV, PJ_J=p-value of t-test between hybrids and *C. jacchus*, PJ_P=p-value of t-test between hybrids and *C. penicillata*. Significant p-values are indicated as "*" for p-value<0.05, "**" for p-value<0.01, and "***" p-value<0.001.

	C. penicillata x *C. jacchus* (PJ)						
Trait	N	Mean	SD	MPV	M	PJ_J	PJ_P
BODY (cm)	54	21.3	2.7	20.4	*	**	0.472
Femur (cm)	54	5.8	0.6	5.6	0.100	0.074	0.921
FO (mm)	49	39.2	3.1	39.8	0.255	0.523	0.259
FOREARM (cm)	54	4.6	0.5	4.5	0.099	0.129	0.498
FOOT (mm)	54	54.4	5.1	54.4	0.932	0.793	0.837
HAND (mm)	50	34.4	3.6	35.3	0.094	0.221	0.330
HUMERUS (cm)	54	4.7	0.7	4.6	0.421	0.756	0.418
IC (mm)	54	28.9	2.2	27.9	**	**	0.229
JAW (mm)	53	23.7	4.2	22.6	0.060	0.073	0.236
TAIL (cm)	52	28.7	2.4	27.5	***	0.069	0.061
TIBIA (cm)	54	6.7	0.6	6.5	0.119	0.495	0.189
WEIGHT (g)	53	317.9	73.2	315.5	0.808	0.766	0.485
ZYG (mm)	53	29.1	2.4	28.6	0.168	0.364	0.235

Table 5 Summary of species means, standard deviations (SD), sample numbers (N), mean mid-parental values (MPV) for thirteen morphological traits in *Callithrix penicillata* x *Callithrix geoffroyi* hybrids. M=p-values from t-tests from hybrid to MPV, GP_G=p-value of t-test between hybrids and *C. geoffroyi*, GP_G=p-value of t-test between hybrids and *C. penicillata*. Significant p-values are indicated as "*" for p-value<0.05, "**" for p-value<0.01, and "***" p-value<0.001

	C. penicillata x *Callithrix geoffroyi* (PG)						
Trait	N	Mean	SD	MPV	M	GP_G	GP_P
BODY (cm)	18	21.4	0.9	21.5	0.557	0.181	0.244
Femur (cm)	18	6.6	0.6	6.1	**	0.233	***
FO (mm)	18	37.7	3.7	40.0	*	*	*
FOREARM (cm)	18	4.8	0.4	4.7	0.480	0.656	0.104
FOOT (mm)	16	53.3	3.2	54.8	0.075	0.081	0.328
HAND (mm)	9	36.8	3.2	35.8	0.360	0.731	0.207
HUMERUS (cm)	18	5.1	0.4	5.0	0.120	0.086	***
IC (mm)	18	29.8	2.2	29.3	0.372	0.575	0.024
JAW (mm)	18	22.7	2.9	24.3	*	**	0.810
TAIL (cm)	18	30.7	1.6	29.2	**	0.979	***
TIBIA (cm)	18	7.1	0.4	6.8	*	0.586	***
WEIGHT (g)	18	355.8	27.8	347.3	0.210	0.111	***
ZYG (mm)	18	30.3	1.2	29.3	**	0.853	***

Table 6 Species mean pairwise genetic distances of four *Callithrix* species based on previously published mitogenomic haplotypes which include a subset of marmosets sampled in this study.

	C. aurita	*C. geoffroyi*	*C. jacchus*	*C. penicillata*
C. aurita				
C. geoffroyi	0.059			
C. jacchus	0.060	0.018		
C. penicillata	0.059	0.018	0.014	

Additional Files

Additional file 1 — Supplementary_Figure_S1.pdf
Pictures showing labeled facial regions used for phenotypic identification of sampled hybrids.

Additional file 2 — Supplementary_Figure_S2.pdf
Morphological variable normal QQ plots for thirteen morphological traits used in this study.

Additional file 3 — Supplementary_Table_S1.tsv
Table S1. Metadata and individual morphological trait measures for sampled marmosets. The 'Individual' column gives ID of each sampled individual. The 'Place of Collection' column indicates whether an individual was sampled in the wild, at a captive facility, or came from the wild and then was transferred to a captive facility. The Guarulhos Municipal Zoo is located in Guarulhos, São Paulo, Brazil; CPRJ (Centro de Primatologia do Rio de Janeiro) is located in Guapimirim, Rio de Janeiro, Brazil; CEMAFAUNA (Centro de Conservação e Manejo de Fauna da Caatinga) is located in Petrolina, Pernambuco; DEPAVE (Prefeitura Municipal de São Paulo, Secretaria Municipal do Verde e Meio Ambiente - DEPAVE (Divisão Técnica de Medicina Veterinária e Manejo da Fauna Silvestre) is located in São Paulo, São Paulo, Brazil; PET (Parque Ecológico do Tiete) is located in São Paulo, São Paulo; PARNASO (Parque Nacional Serra dos Órgãos) is located in Teresopolis, Rio de Janeiro, Brazil. SERCAS (Setor de Etologia aplicada à Reintrodução e Conservação de Animais Silvestres) is located in Campos dos Goytacazes, Rio de Janeiro, Brazil. The 'City' and 'State' columns indicated where each individual was sampled. Abbreviations for Brazilian states in the 'State' column are as follows: Espírito Santo (ES), Minas Gerais (MG), Pernambuco (PE), Rio de Janeiro (RJ), São Paulo (SP). The 'Taxon' column indicates whether the sampled individual possessed a pure species or hybrid phenotype. Taxon abbreviations in this column are as follows: 'A' is *C. aurita*, 'G' is *C. geoffroyi*, 'J' is *C. jacchus*, 'P' is *C. penicillata*, 'AH' is *C. aurita* hybrid , 'PJ' is *C. jacchus* x *C. penicillata* hybrid, 'PG' is *C. penicillata* x *C. geoffroyi* hybrid, and 'CC' is *Callithrix* sp. x *Callithrix* sp. hybrid. The 'Sex' column indicates the sex of the sampled individuals (F=Female, M=Male). The 'Age' column indicates the age of the sampled individual (A=Age). The rest of the columns show individual measures for thirteen morphological traits (NA=No data Available). Abbreviations in each trait column match those described in the methods. Traits with left and right measures have been averaged for the analyses described in the methodology section of the main text.

Additional file 4 – Supplementary_Table_S2.tsv
Supplementary Table S2. List of previously published mitogenome haplotypes used to calculate genetic distances between the four marmoset species included in this study.

Additional file 5 – Supplementary_Table_S3.tsv
Supplementary Table S3. Results of univariate Welch's ANOVA test for differences across all *Callithrix* taxa for 13 morphometric traits.

Additional file 6 – Supplementary_Table_S4.tsv
Supplementary Table S4. Games-Howell post-hoc pairwise tests after Welch's ANOVA to determine which comparisons between *Callithrix* taxa for thirteen individual traits are significant. 'Trait' column names of traits follow that of Supplementary Table S1. 'Group 1' and 'Group2' indicate which two taxa are being compared and abbreviations follow Supplementary Table S1. 'Estimate' column refers to the mean difference between the groups being compared, 'conf.low' column refers to lower limit of the confidence interval for the mean difference, 'conf.high' column refers to higher limit of the confidence interval for the mean difference, 'p.adj' is the adjusted p-value using Turkey's method, and 'p.adj.signif' column indicates the significance level of adjusted p-values with 'ns' meaning note significant.

Additional file 7 – Supplementary_Table_S5.tsv
Supplementary Table S5. Eigenvalues and variance of principle components (PCs) for *C. jacchus* and *C. penicillata* hybrids and parental species.

Additional file 8 – Supplementary_Table_S6.tsv
Supplementary Table S6. Loadings of principal components (PCs) for *C. jacchus* x *C. penicillata* hybrids and parental species

Additional file 9 – Supplementary_Table_S7.tsv
Supplementary Table S7. Eigenvalues and variance of principle components (PCs) for *C. geoffroyi* and *C. penicillata* hybrids and parental species.

Additional file 10 – Supplementary_Table_S8.tsv
Supplementary Table S8. Loadings of principal components (PCs) for *C. geoffroyi* x *C. penicillata* hybrids and parental species

Additional file 11 – Supplementary_Table_S9.tsv
Supplementary Table S9. Eigenvalues and variance of PCs (principle components) for *C. aurita*, *C. jacchus*, *C. geoffroyi* and *C. penicillata* hybrids and parental species.

Additional file 12 – Supplementary_Table_S10.tsv
Supplementary Table S10. Loadings of PCs for *C. geoffroyi*, *C. penicillata*, *C. jacchus*, and *C. aurita* hybrids and parental species.

Additional file 13 – Supplementary_Figure_S1_legend.txt
Figure legend for Supplementary Figure S1.

Additional file 14 – Supplementary_Figure_S2_legend.txt
Figure legend for Supplementary Figure S2.