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New developmental stages for common marmosets (*Callithrix jacchus*) using mass and age variables obtained by K-means algorithm and self-organizing maps (SOM)

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ABSTRACT

This study proposes new developmental stages for *Callithrix jacchus*, using K-Means algorithm and an artificial neural network–self-organising maps (SOM) as computational tools, based on weight and age. Eight developmental stages are proposed: Infant I, II and III, Juvenile I and II, Sub adult, Young adult and Older adult. This classification is consistent with the first appearance of several behavioural and physiological characteristics and thus may have generality in defining critical developmental periods. It also reveals differences in male and female development and establishes a stage for the onset of the final adult life cycle. This classification is also important to understanding the biology of the ontogenetic development of common marmosets, providing new insights for the management and care of captive animals and improving age estimate indicators when specimens are captured in long term monitoring of free ranging groups.

1. Introduction

Biomedical research using the common marmoset (*Callithrix jacchus*) continues to grow, mainly in infectious diseases, neuroscience and development, toxicology and drug development, reproductive biology and behavior [19]. The life span of this species is around 11.7 years (Rowe, 1996). A strong preventive medicine health care program and diagnostic services are critical to maintaining a healthy, robust colony [18]. The use of common marmosets in biomedical research started in the early sixties and information about free-ranging groups began to appear in the early seventies. One of the first studies focusing on the ecology and behavior of genus *Callithrix* [23] using behavioral data identified six developmental months), Adolescent (6–10 months), Sub adult (11–15 months), and Young adult (> 15 months). Another classification was proposed by Yamamoto [27] based on behavioral data and age, as follows: Infant (0–5 months), Juvenile (5–10 months), Sub adult (10–15 months) and Young adult (> 15 months). In a study where the weights of captive and wild common marmosets were compared at the different age ranges proposed by Yamamoto [27], Araújo et al. [3] found that the profile of both groups increases similarly with no statistical differences between age ranges. Recently, Abbott et al. [2], describing some aspects of common marmoset developmental biology, considered the infant stage as ranging from 0 to 3 months, Juvenile from 3 to 12 months, Sub adult from 13 to 18 months and Adult over 18 months.

In the last decade biomedical and computer engineering research have explored artificial neural networks (ANN). Articles in this area have tripled in the last five years [17]. Examples include sorting and allocating upper and molar teeth of primates [20] monitoring wolf preserves in Portugal [4], recognition patterns and monitoring of ecological data [28], special patterns of behavior of non-native fish species [6], simulation of anxiety-like behavior in rats [21], predicting the extent of tumors in men with clinically localized prostate cancer [9], and clinical decision support systems for intensive care units [8], among others. Despite the potential use of common marmosets in field studies and biomedical research, particularly in experimental protocols where the variables "weight" and "age" are critical, the classification of development, ontogenetic stage or relative age needs to be precise to refine data analysis. For instance, according to current classifications, mean weight for the Infant stage is around 139 g when the weight of newborn babies shows mean values around 30 g, and there are no objective criteria for distinguishing between "newborn" and "infant." Also, a more appropriate method for classifying the juvenile stage of *C. jacchus* seems to be needed; this will be useful for both field studies and experimental procedures requiring precise weight control. The objective of this study was to provide a developmental classification for common marmosets using weight and age variables analyzed by two

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Table 1
Significant clusters found by K-means and SOM to classify the *C. jacchus* developmental stages regarding weight and age (M = males, F = females).

Clusters	New classes	Weight (g) Mean ± SD	Age (days) Mean ± SD	Number of records
1	INF1	40.07 ± 11.79	13 ± 13	M = 234; F = 235
2	INF2	97.88 ± 26.12	67 ± 20	M = 264; F = 272
3	INF3	165.38 ± 20.40	118 ± 19	M = 319; F = 356
1	JUV1	197.68 ± 35.60	190 ± 27	M = 391; F = 424
2	JUV2	255.43 ± 33.78	259 ± 26	M = 153; F = 144
1	SAD1	259.54 ± 42.89	343 ± 26	M = 150; F = 142
2	SAD2	307.97 ± 40.31	408 ± 28	M = 343; F = 373
1	ADU1	311.00 ± 44.40	829 ± 300	M = 1741; F = 1523
2	ADU2	374.63 ± 45.03	1534 ± 582	M = 1078; F = 1058

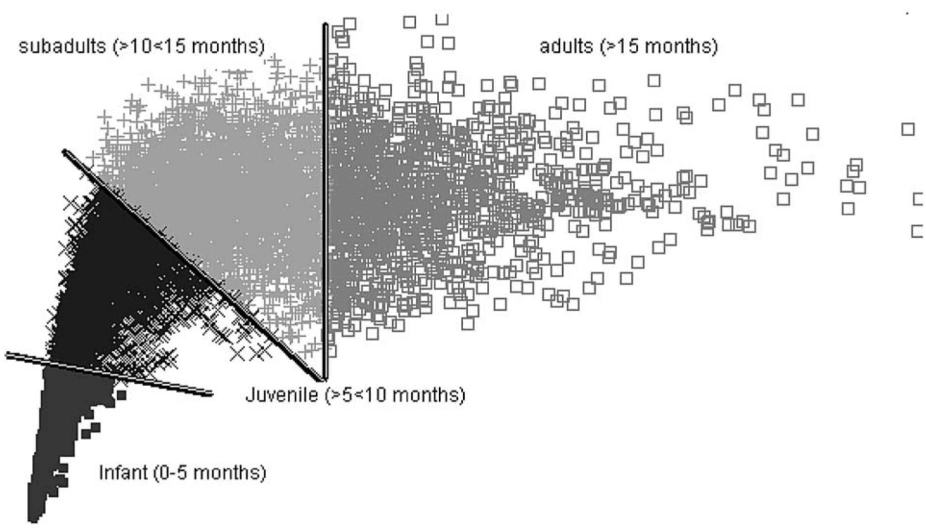

Fig. 1. Database split into four datasets using the age stages proposed by Yamamoto [26].

computational approaches: K-means algorithm and an artificial neural network–self-organizing maps (SOM).

2. Methods

2.1. Subjects

We used a database with 9,200 weight entries recorded across the ontogenetic development of *C. jacchus* from 1985 to 2003 (see Table 1), all data used in this study came from a born captive measurements of weight and age. During this time the number of entries over 4-year periods were: 1985–1988: $n = 991$; 1989–1992: $n = 4,097$; 1993–1996: $n = 1919$; 1997–2000: $n = 1931$ and, in the last three years, $2001 = 200$, $2002 = 28$ and $2003 = 34$. The animals were housed in outdoor cages, at the Núcleo de Primatologia of the Universidade Federal do Rio Grande do Norte ($5^\circ\ 50'$S $35^\circ\ 12'$W).

The database was built in templates using ACCESS MS ™ and imported to JMP statistical software, version 5.0.1a—Copyright© 1989–2002 SAS Institute Inc. All statistical tests, K-means and self-organizing maps were used in the JMP routines. We excluded 761 records related to two situations: (1) when the weight of pregnant females reached 410 g, which corresponds to mean weight plus standard deviation values obtained for the males of the colony; (2) the weight of males and females who experienced chronic weight loss and the weight of dying animals. In this study we used the age in days proposed by Yamamoto [27], as follows: Infant class: 0–150 days; Juvenile: 151 to 300 days; Sub adult: 301–600 and Adult: > 600 days.

2.2. Computational techniques

Clustering is one technique that falls into a group of undirected data mining tools. The goal of undirected data mining is to discover structure in the data as a whole. There is no target variable to be predicted, thus no distinction is made between independent and dependent variables. Both K-means algorithm and self-organizing maps [16] are clustering methods that classify patterns without the need of previous information on data distribution, as occurs in supervised methods. These techniques allow a data mining process that identifies stages and sub-stages of the data distribution being used. Thus, these tools are better for modeling and identifying stages in a sample pool and they enable us to visualize data distribution in a two dimensional space, even when the data form high dimensional spaces, i.e. when the input vector is high. These techniques are effective analytical tools for extracting information from a large dataset, such as that used in this study, and are more suitable than traditional statistical methods (such as regression) for detecting multivariate data patterns.

2.3. Data mining

The aim of data mining is to extract non-explicit information, that is, patterns that may be labelled as a database class [10,13] Cluster-identifying techniques are useful tools for identifying and classifying patterns immersed in a mass of data.

Two techniques were used in this study: the K-means algorithm [7] and the self organizing maps, Kohonen [16]). Two algorithms

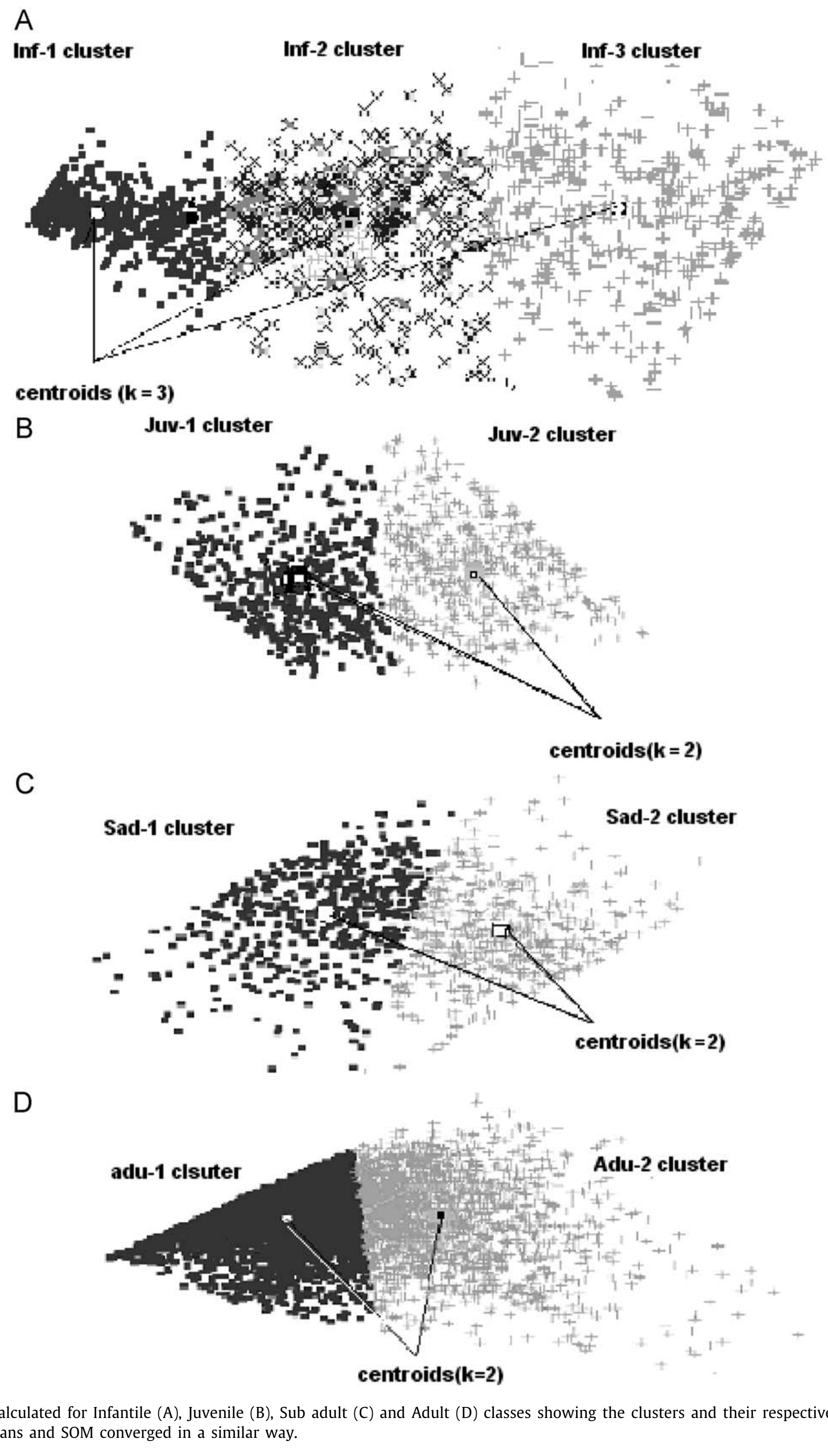

Fig. 2. Classification map calculated for Infantile (A), Juvenile (B), Sub adult (C) and Adult (D) classes showing the clusters and their respective *centroids* (k) for each class. In both distributions, K-means and SOM converged in a similar way.

were chosen in order to approach the problem in two ways and verify if the methods converge to a same solution. Both algorithms (K-means and SOM) are able to accurately find the clusters.

The K-means method introduced by Duda et al. [7] is one of the simplest and most efficient cluster techniques, consisting of a self-organized identification process of the centroid of each cluster starting from a set of mobile center candidates. The mobile centers are initialized and using a measure of similarity that measures the distance of each of the patterns to the centers, selects the nearest center as the winner, which, by means of an adaptative process, is

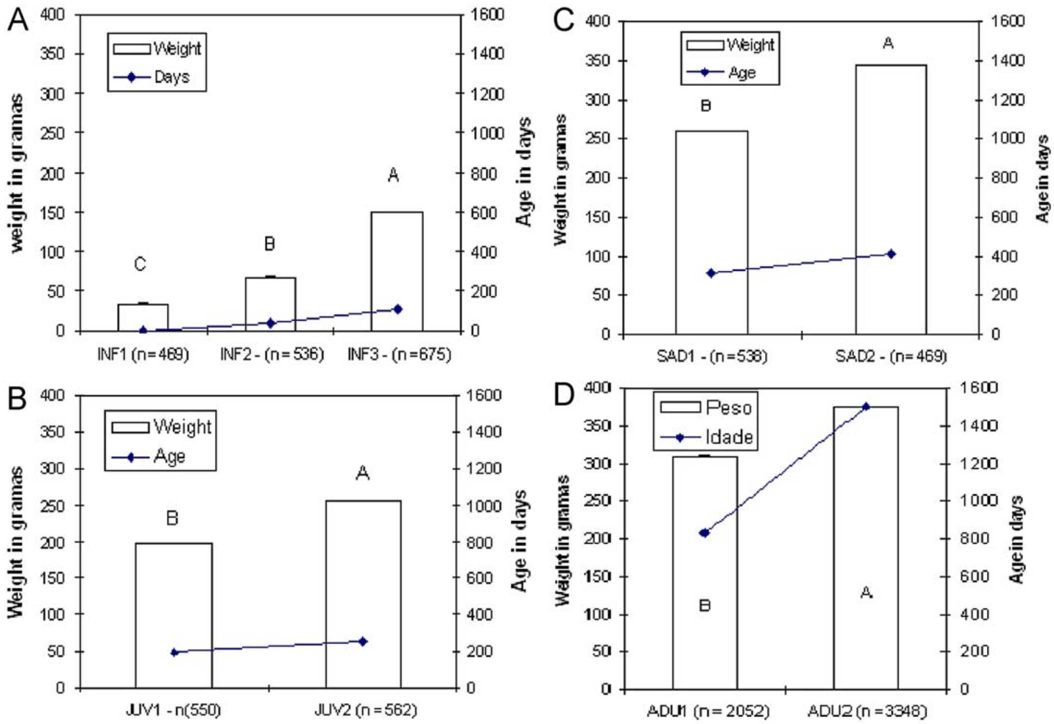

Fig. 3. Developmental stages found using weight and age variables by K-means and SOM analysis for common marmosets, based on classification proposed by Yamamoto [26]. Bars indicate weight, and the line indicates the age of the individuals. (1A) Three sub-stages were found for the infant stage; (1B) two sub-stages were found for the juvenile stage; (1C) two sub-stages were also found for the sub adult stage and (1D) one stage was found for adult. In all cases, differences between sub-stages were significant using Student's t-test (a,b) or ANOVA (a,b,c,), $p < 0.001$ for both.

able to move towards the pattern (a point in the data space). The process is concluded when each mobile center reaches a position at the center of a cluster. Once the clusters are identified, the labeling process is carried out and the patterns are classified. The process can be seen as a winner-take-all competitive process, which may be implemented as a competitive neural network.

The self-organizing map algorithm is an unsupervised neural network developed by Kohonen [16]. This neural network is usually a uni- or bi-dimensional lattice. These neurons receive the input data and compete according to the measure of the distance between their synaptic weights and the input patterns. The weight vector closest to the input vector is chosen as the winning neuron. Each winning neuron establishes a cooperative and selective process with neighbouring neurons to form neuron clusters. The process continues until neuron distribution in the grid is self-organized. At the end of the process the neuron clusters that reflect the same unknown organization of the input data or input network patterns are identified. From these clusters it is possible to label and classify the patterns.

2.4. Choice of the right number of clusters

If the number of clusters k in the K-means method is not chosen to match the natural structure of the data, the results cannot be accurate. In this study, the proper way to avoid this is to experiment with different values for k. In principle, the suitable k value will have the shortest intra-cluster (the distance between two inputs of same cluster) and longest inter-cluster distances (the distance between two inputs of different clusters). Once the clusters are discovered, they have to be interpreted in order to have some value for the data-mining project [13].

To perform this option we develop a heuristic based on the knowledge of the intrinsic characteristics of the problem and the number of clusters chosen matches the biological interpretation of the data. First we test each group, using k values between 2 and 6, and the statistical separation for each. The suitable k value that shows the highest statistical separation is chosen.

3. Results

In this study we observed for a same database that the K-means algorithm and the SOM algorithm converged to a same result, enabling the process of classification by age and weight, thus meeting the objective of this study.

Using the number of age stages proposed by Yamamoto [27], we divided our databank into 4 classes by K-means, as shown in Fig. 1. Additionally, the dataset was split using the Yamamoto classification. Within each class, separately, we performed new data mining using the K-means and SOM algorithm.

For the infant group a different number of k values (k = number of possible clusters) was used to find the suitable classification. K-means classification and SOM produced the results that matched biological observations for the infant group ($k = 3$), since the *centroids* (which equal the mean of each cluster), showed three classes, INF1, INF2 and INF3 (INF = cluster—see Table 1, Fig. 2A) the same mean values for *weight* and *age* were found for the centroids in both K-means and SOM classifications. When a statistical analysis of bivariate distribution routine was used (age and weight) a strong correlation was found between the two variables (weight and age-Pearson, $r = 0.937$, $p < 0.001$).

The same computational approaches were used to test if different k numbers (clusters parameters) were present in the other age ranges: juvenile, sub adult and young adult. In all the three age stages, two sub-classes were found as follows: JUV1 and JUV2 (Fig. 2B), SUB1 and SUB2 (Fig. 2C) and ADU1 and ADU2 (Fig. 2D), respectively. In the Juvenile and Sub adult age classes tested, the

Table 2
New proposed classification for ontogenetic development of *C. jacchus*, and first main behavioural manifestation or cessation during ontogenetic development.

New stages	Weight (g)	Age (days)	Age range in months	Behavioural correlates (first manifestation)
Infantile I	40.07+11.79	13+13	0–1	Off episodes; scent marking
Infantile II	97.88+26.12	67+20	1–3	Vocalisation; piloerection-wrestling play with "open mouth face"; independent locomotion; solid ingestion; self feeding
Infantile III	165.38+20.40	118+19	3–4	Social play; complete weaning
Juvenile I	197.68+35.60	190+27	4–7	Onset of puberty in females; social grooming
Juvenile II (adolescent)	255.43+33.78	259+26	7–10	Onset of puberty in males
Sub adult	307.97+40.31	408+28	10–15	Ovulation; copulation
Young adult	374.63+45.03	1534+532	15–72	Reproduction
Older adult	352.77+46,61	2116	> 72	Hearing loss; cartilage aging; aging

Behavioural information was obtained from Ingram [12], Abbott [1], Yamamoto [27], Ximenes & Sousa [26], Hearn [14], Tardif and Jaquist [22] and Abbott et al. [2].

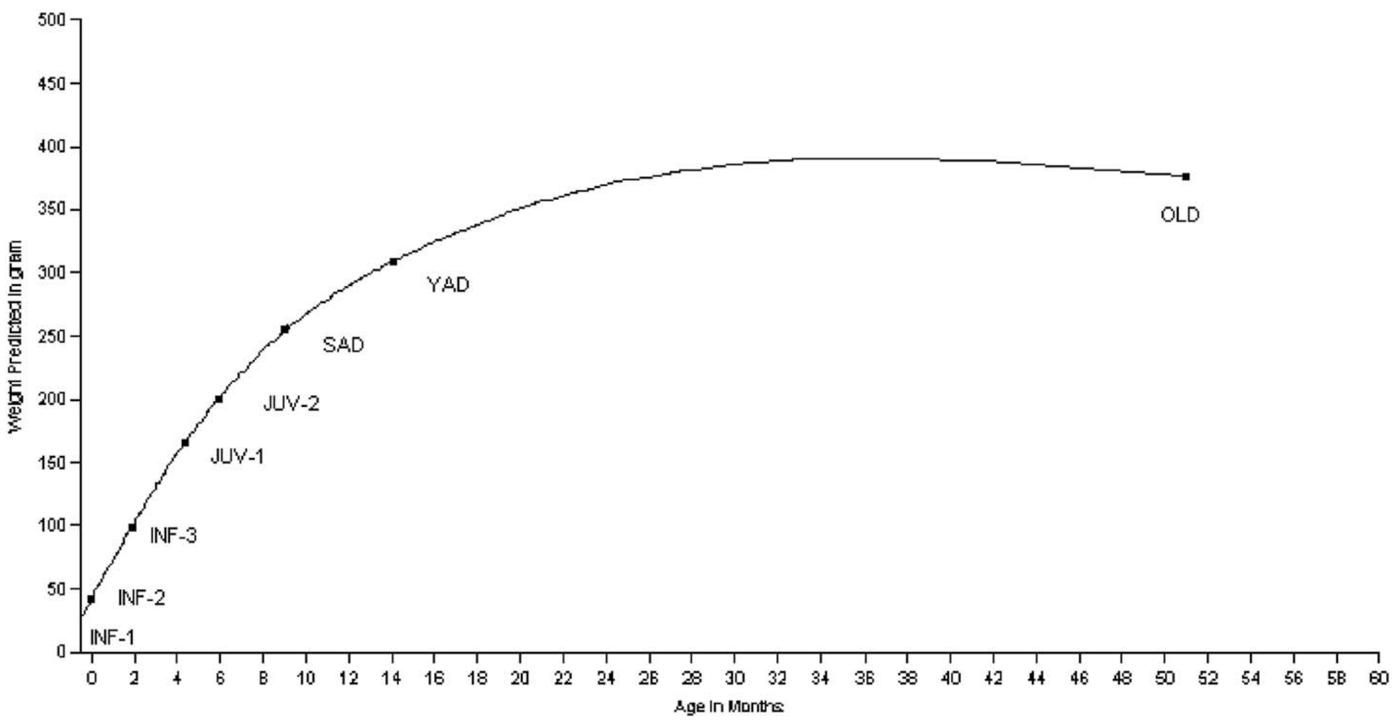

Fig. 4. Weight prediction curve (WPC) for the ontogenetic development of *C. jacchus* using weight and age calculated by SOM and K-means analyses.

variables *weight* and *age* showed the same increase pattern, but the correlation found using a bivariate statistical test, showed a weak correlation between *weight* and *age* for both classes (Pearson, Juvenile: $r = 0.466$, $p < 0.05$; Sub adult: $r = 0.219$, $p < 0.05$). Weight was highest in the adult stage, and continued to vary with a standard deviation of ± 40 g, while age increased steadily until the animal died. The correlation between weight and age using statistical bivariate distribution was weak (Pearson, $r = 0.152$, $p < 0.05$) for the adult classification, as illustrated in Fig. 3.

Table 1 shows the class transitions. In spite of what was found, statistical differences in all classes showed that the JUV2 stage mean (255.43 g) was very similar to that of the SAD1 stage (259.54), as were those between the SAD2 (307.97 g) and ADU1 stages (311.00 g). Since no statistical differences were found between SAD1 and ADU1, they were disregarded, leaving only one class for both stages. The final classification results using SOM and K-means analysis, with eight developmental stages and their range in months, are shown in Table 2, as well as the behavioral correlates of these stages obtained from the literature. Using the proposed classification, a prediction weight curve (PWC) was calculated (Fig. 4), where maximum weight is plotted between the SAD and ADU stages. The PWC corresponds to the weight gain of the animal during ontogenetic development. It was obtained from regression using both weight *x* age variables. As can be observed, there is a very rapid weight increase in the first 7 months of development, reaching about 75% of adult weight when the individual is in the JUV2 stage. During the adult stage, weight stabilization occurs, with a slight decrease at around four years of age. The older adult stage starts at 72 months.

4. Discussion

This study reveals that there are natural weight classes or developmental phases in *C. jacchus* that are not evident when analyzing weight data under the assumptions of standard statistical or growth models. Other species may also show such pattern. Age and weight are the most frequently monitored variables in animals living in breeding colonies and zoos, as well as in long-term studies of free-ranging animals when they are captured and characterized biometrically and medically. We demonstrate the use of a K-means algorithm classification and an artificial neural network–self-organizing maps (SOM) that may also be useful in assessing developmental stages in various animal species. Moreover, it opens the possibility that other variables, such as cephalic or thoracic perimeters and knee-to-heel length of long bones, which are also used for ontogenetic classifications [12], will be revised based on these computational tools. According to the present findings, it is proposed that in *C. jacchus* the conventional infant stage should be divided into three sub-stages: Infant 1: 0–30 days of age (1st month), with mean weight of 40 g (SD $\pm$ 11.79 g); Infant II: age greater than 30 and less than 90 days (2nd–3rd months), with mean weight of 98 g (SD $\pm$ 26 g) and Infant III: age greater than 90 and less than 120 days (4th month), with mean weight of 165 g (SD $\pm$ 20 g). The juvenile stage can be divided into two sub-stages: Juvenile I: age from 120 to 210 days (5th–7th month), with mean weight of 197.68 g (SD $\pm$ 36 g) and Juvenile II: age between 210 and 300 days (7th–10th month), mean weight of 255.43 g (SD $\pm$ 34 g). The sub adult stage is from day 300 to 450 (11th–15th month), mean weight of 307.97 g (SD $\pm$ 11.79 g). The adult stage begins at 450 days (16th month) and extends up to 2116 days (around 6 years), with mean weight of 374 g (SD $\pm$ 11.79 g). The older adult stage begins at 6 years and ends at death.

This new ontogenetic classification for the common marmoset includes eight stages, based on increased age and weight during the animal's development. It should be pointed out that the classification proposed is similar to others, such as that of Yamamoto [27], since both establish the end of the juvenile and sub adult phases at 10 and 15 months of age, respectively.

Behavioral patterns associated with the development of *C. jacchus* are found in various publications ([1,2,5,14,23,25,26,27] among others). According to these studies, behaviors related to carrying, nursing, feeding, play and agonism appear for the first time during the infant stage. According to the revised developmental classification proposed in the present study, in which the infant stage has three sub-stages, the first episodes of piloerection coincide with the onset of the infant II stage, and weaning is completed in the transition between the Infant II and Infant III stages [24]. Furthermore, females show increased estradiol during the Juvenile I stage, while the males present increased testicular size and elevated testosterone levels in Juvenile II stage [2]. These results, therefore, allow definition of a more precise and functional developmental scale, since the onset and end of these activities were not clearly associated with any developmental indicators using the conventional scale. For example, the complete weaning that occurs around the 14th week [12,26], approximately five weeks before the end of the infant stage in Yamamoto's [27] classification, now coincides with the end of the Infant III stage. The statistical differences found in the infant and juvenile stages are explained by the pronounced weight gain of around 75% between the first and tenth months of life when compared to the weight of an adult animal.

Similar results had already been reported for *Saguinus fusciollis* [15]. On the other hand, in the sub adult and adult stages, only the final increase to adult weight was observed, with a mean variation of $\pm$ 40 g until the end of the animal's life. In a study by Abbott [1] on physical, hormonal and behavioural development of *C. jacchus*, based on the weight profile of 20 males and 20 females born in captivity, an ascending progression can be observed in weight gain. This coincides with our infant stage period and, to a lesser degree, the Juvenile I and II stages of the present classification. According to Abbott, the captive animals gained the same weight as the wild animals present in the colony (300 $\pm$ 6.6 g) at between 500 and 550 days of development (16 to 18 months), which corresponds to the adult stage.

According to Tardif et al. [25] the average lifespan of a *C. jacchus* that survives infancy is approximately 6 years and the maximum life expectancy of *C. jacchus* is around 15 years in captivity. In a study on the biological and developmental aspects of *C. jacchus*, Abbott et al. [2] showed that from the age of 7–8 years, they had more diseases, greater hearing loss and cartilage aging. In the present study, 5.7 years was considered the onset of weight loss and the beginning of aging.

Through the stages found in this study, results show that a prediction weight curve (PWC) can be created and successfully used. The use of PWC could be of great value in following the ontogenetic development of *C. jacchus* and in selecting subjects for chronic experiments with greater certainty, since weight profile could indicate a tendency for developing Wasting Marmoset Syndrome (WMS), which is a significant cause of death in captive callithrichids [11]. Animals with the potential for developing WMS generally have weights below the curve, beginning in the juvenile stage. PWC could also be used to classify the age of animals in field studies, in addition to morphometric measures already being used.

5. Summary

Common marmoset (*Callithrix jacchus*) is one of the most investigated species of New World primate in different areas of biomedical research such as infectious diseases, neuroscience and development, toxicology and drug development, reproductive biology and behavior. Therefore, a precise developmental classification, particularly in experimental protocols where the variables "weight" and "age" are critical, are necessary to refine data analysis. According to current classifications, based on age and behavioral variables, mean weight for the infant stage is around 139 g when the weight of newborn babies shows mean values around 30 g, and there are no objective criteria for distinguishing between "newborn" and "infant." Also, a more appropriate method for classifying the juvenile stage of *C. jacchus* seems to be needed since major physiological changes, particularly related do reproductive maturation, occurs at this stage. This study proposes a redefinition of developmental stages based on age and weight variables using a large series of colony-bred animals. We used K-Means and the artificial neural network–self-organizing maps algorithms to analyze the data. The goal of undirected data mining is to discover structure in the data as a whole. Both K-means algorithm and self-organizing maps are clustering methods that classify patterns without the need of previous information on data distribution, as occurs in supervised methods. These techniques are effective analytical tools for extracting information from a large dataset, such as that used in this study, and are more suitable than traditional statistical methods (such as regression) for detecting multivariate data patterns and allow a data mining process that identifies stages and sub-stages of the data distribution being used. Thus, simple clustering techniques involve modeling and identifying stages in a sample pool, enabling us to visualize data distribution in a two dimensional space, even when the data form high dimensional spaces, i.e. when the input vector is high. We found for a same database that both approaches converged to a same result, enabling the process of classification, thus reaching the goal of this study. This will be useful for both experimental procedures and field studies requiring precise weight control. Our results indicate that infantile and juvenile stages can be divided, respectively, into Infant-1, Infant-2 and Infant-3 periods and Juvenile-1 and Juvenile-2 stages. The revised tripartite stages on infancy are coincident with the onset of a variety of behavioral, physiological and hormonal indicators that have heretofore not been recognized as being so tightly constrained by size and age. Results also show differences in male and female development and ascertain a stage for the onset of the final adult life cycle. According the results, a prediction weight curve (PWC) can be created and successfully used to classify the age of free-ranging animals, in addition to morphometric measures previously being used.

Conflict of interest statement

None declared.

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