Instituto Nacional de Pesquisas da Amazônia - INPA
Programa de Pós-Graduação em Ecologia

Dieta do *Plecturocebus cinerascens* (Platyrrhini: Primates): a influência da composição química na escolha de frutos consumidos

Fábio de Souza Mattos

Manaus – AM
Agosto, 2016

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Dissertação apresentada à Coordenação do Programa de Pós-Graduação em Ecologia do Instituto Nacional de Pesquisas da Amazônia, como requisito regulamentar obrigatório para obtenção do título de Mestre em Biologia (Ecologia).

Manaus – AM
Agosto, 2016

Banca examinadora da defesa oral pública

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Sinopse:

Neste trabalho foi avaliada a influência de metabólitos secundários (taninos e alcaloides) no consumo de frutos por primatas da espécie *Plecturocebus cinerascens* (Platyrrhini: Pitheciidae). Este, que foi o primeiro estudo ecológico da espécie, foi realizado na área da Usina Hidrelétrica Rondon II, município de Pimenta Bueno, Rondônia, Brasil. Relacionou-se a concentração de taninos e alcaloides dos frutos com o peso das partes consumidas e descartadas por *P. cinerascens* em cada espécie de planta.

Palavras-chave: zogue-zogue-cinza, *Plecturocebus cinerascens*, primatas, frutos consumidos.

Dedicatória

Dedico este trabalho à minha esposa, por todo apoio e companheirismo, e aos meus pais, pelos exemplos de vida e ensinamentos que me permitiram chegar até aqui.

Agradecimentos

Resumo

As plantas se protegem de herbívoros através de vários mecanismos mecânicos e químicos de defesa. Grande parte dos metabólitos secundários que as plantas produzem, como taninos e alcaloides, são apontados como impedidores de consumo para herbívoros. É possível que a quantia de massa ingerida de frutos consumidos por *Plecturocebus cinerascens* (Platyrrhini: Pitheciidae) seja menor em função a concentração e do tipo de metabólitos secundários (taninos e alcaloides) contidos nos frutos. Meu trabalho de campo foi realizado na área da Usina Hidrelétrica Rondon II, Pimenta Bueno, Rondônia. Para registrar as espécies de frutos consumidos por *P. cinerascens* eu realizei buscas pelos animais em sete locais diferentes, sendo, cada local, a área de vida de um grupo de zogue-zogues-cinza distinto. Uma vez registrada a alimentação de um determinado grupo de zogue-zogues, eu realizava a busca por um grupo diferente com o intuito de evitar pseudo-repetição amostral. Coletei amostras de frutos para análise química em laboratório em todas as plantas em fase de frutificação onde os animais se alimentaram. A análise química foi realizada tanto nas partes consumidas, quanto nas partes não consumidas dos frutos. O resultado da regressão múltipla não apontou relação entre a concentração de metabólitos secundários e o peso de massa úmida dos frutos consumidos pelos zogue-zogues-cinza. Contudo, os animais descartaram as partes dos frutos nas únicas espécies de plantas (*Amorimia* [*Mascagnia*] *rigida* [Malpighiaceae] e *Inga* sp. [Fabaceae: Mimosoideae]) que apresentaram alcaloides. Concluo que a alimentação do *P. cinerascens* não é influenciada por metabólitos secundários (taninos e alcaloides) e indico investigação mais ampla sobre a composição química dos itens alimentares que *P. cinerascens* consome para avaliar se outros compostos químicos, não avaliados neste estudo (como: proteínas, lipídios, açúcares, entre outros), influenciam a dieta destes primatas.

Abstract

Influence of tannins and alkaloids on the choice of fruits eaten by
***Plecturocebus cinerascens* (Platyrrhini: Pitheciidae)**

Plants protect themselves against herbivores in many mechanical and chemical defense mechanisms. Much of the secondary metabolites that plants produce, e.g. tannins and alkaloids, have been shown to deter herbivores. It is possible that the amount of ingested mass of fruits consumed by *Plecturocebus cinerascens* (Platyrrhini: Pitheciidae) is lower depending on the concentration and type of secondary metabolites (tannins and alkaloids) contained in the fruits. My fieldwork was carried out in the area of the Rondon II Hydroelectric Power Plant, Pimenta Bueno, Rondônia State, Brazil. In order to record the species of fruit consumed by *P. cinerascens*, I searched the animals at seven different sites, each site being the living area of a distinct Ashy titi monkey group. Once I registered the feed of a certain group of Ashy titis, I was searching for a different group in order to avoid sample pseudo-repetition. I collected fruit samples to perform chemical analysis in all plants in the fruiting stage where the animals were fed. The chemical analysis was performed both in the consumed parts and in the unconsumed parts of the fruits. The results of the multiple regression did not show a relation between the concentration of secondary metabolites and the weight of the freshy mass of the fruits consumed by Ashy titi monkeys. However, the animals discarded the parts of the fruits in the only plant species (*Amorimia* [*Mascagnia*] *rigida* [Malpighiaceae] and *Inga* sp. [Fabaceae: Mimosoideae]) that presented alkaloids. I conclude that the diet of *P. cinerascens* is not influenced by secondary metabolites (e.g. tannins and alkaloids) and indicates a broader investigation of the chemical composition of food items that *P. cinerascens* consumes to evaluate if other chemical compounds, not evaluated in this study (e.g. proteins, lipids, sugars and others), influence the diet of these primates.

Sumário

1. Introdução

Primatas de vida livre precisam lidar com vários fatores externos que podem influenciar sua dieta. Fatores como a disputa por recursos existentes em uma dada área [Dew, 2005; Ganzhorn, 1988; Palminteri et al., 2013], a disponibilidade de alimentos necessária para suprir sua demanda diária de energia [Heiduck, 1997; Mourthé, 2012; Stevenson et al., 2000; Souza-Alves et al. 2011; Stevenson, 2001; Worman & Chapman, 2006; Wrangham et al., 1998], assim como a defesa química das plantas das quais se alimentam [Coley et al., 1985; Schmitz & Ritchie, 1991] podem significar o sucesso ou o fracasso em habitar um determinado ambiente [Wright, 1989].

Dentre os fatores citados, a defesa química das plantas através de substâncias tóxicas (metabólitos secundários) [Coley et al. 1985] tem sido alvo de diversos estudos por conta seu poder letárgico [Freeland & Janzen, 1974; Schmitz & Ritchie, 1991], por sua impalatabilidade [Freeland & Janzen, 1974; Hartmann, 1991; Lev-Yadun et al., 2009; McKey et al., 1981; Oates et al., 1980], por desencadear disfunções intestinais [Freeland et al., 1985] e até mesmo por poder ser mortal para os animais [Becker et al., 2013; Bezerra et al., 2012; Duarte et al., 2013; Freeland & Janzen, 1974; Lee et al., 2014; Peixoto et al., 2010]. Para primatas, há estudos que relatam que os metabólitos secundários não exercem influência em sua alimentação [Chapman & Chapman, 2002; Li et al., 2015; Wrangham et al., 1998], assim como estudos que apontam, não só a influência [Emerson & Brown, 2015; Kinzey & Norconk, 1993; McKey et al., 1981], como a adoção de estratégias para evitar a ingestão de toxinas [Corlett & Lucas, 1990].

Entre as quatro famílias de primatas neotropicais (Aotidae, Atelidae, Cebidae e Pitheciidae), os pithecídeos (*Callicebus, Cheracebus* e *Plecturocebus* [Callibinae]; *Cacajao, Chiropotes* e *Pithecia* [Pitheciinae]) são conhecidos como predadores de

sementes [Norconk, 2013]. Em geral, os pithecídeos manipulam os frutos para ter acesso e consumir partes específicas deles (sementes, arilos, polpas) [Ayres, 1986; Barnett, 2010; Boubli, 1999; Bowler, 2007; Norconk et al., 2009; van Roosmalen et al., 1988; Veiga, 2006]. O processo de manipulação é resultado do desenvolvimento de características morfológicas e habilidades que dão a estes primatas a possibilidade de explorar recursos alimentares como frutos duros, que são pouco aproveitados por outras famílias de primatas neotropicais [Ayres, 1986; Barnett, 2005; Boubli, 1999; Boyle, 2008; Kinzey & Norconk, 1990; Norconk, 2011; Norconk, 2013; Norconk & Conklin-Brittain, 2004; Norconk et al., 2009; Palminteri et al., 2013; Veiga, 2006].

O *Plecturocebus cinerascens* [*sensu* Byrne et al., 2015], assim como outras espécies de zogue-zogues [para alguns exemplos, ver: Alvarez & Heymann, 2012; Bicca-Marques & Heymann, 2013; Heiduck, 1997; Souza-Alves et al., 2011], está entre as espécies que consomem os frutos menos duros dentre os pithecídeos devido ao seu porte e morfologia do crânio e da dentição [Norconk et al., 2009]. Muitas vezes estes primatas gastam certa quantia de energia abrindo um fruto, consomem pequenas quantidades de massa e descartam as sementes, que são mais ricas em nutrientes e lipídios [Norconk & Conklin-Brittain, 2004]. Outro fato intrigante é que em frutos de algumas espécies de plantas, como os de *Bellucia grossularioides* (Melastomataceae), o *P. cinerascens* consome aproximadamente metade do fruto e descarta o resto, buscando outro fruto intacto logo em seguida para comer [observação pessoal].

Conforme a teoria do forrageamento ótimo [MacArthur & Pianka, 1966], os animais buscam estratégias para obter energia [Palminteri et al., 2016] com o mínimo de gasto possível [Altmann, 2006; Garber, 1987; Kurland & Beckerman,

1985; Stacey, 1986]. Então, como poderíamos explicar o fato de os animais desperdiçarem recursos depois de terem gasto sua energia para acessá-los? Além de ser pouco conhecida cientificamente, até o momento não houve estudos que buscassem explicar o porquê de tal desperdício por *P. cinerascens*. Assumindo que o processo de manipulação dos frutos e a ingestão de massa apenas de partes específicas dos frutos comidos pelos zogue-zogues podem ser resultado da influência da composição química, meu trabalho tem o objetivo de avaliar a influência da composição química no consumo de frutos por *P. cinerascens*.

Consequentemente, nesse trabalho foi testada a hipótese de que o consumo de frutos por *P. cinerascens* é influenciado pela existência e concentração de metabólitos secundários (taninos e alcaloides), onde: a presença e/ou a concentração de taninos e alcaloides faria os animais consumirem menos massa dos frutos com maior concentração desses compostos.

2. Objetivos

2.1. Objetivo geral

Avaliar a influência de metabólitos secundários (taninos e alcaloides) no consumo de frutos por zogue-zogues-cinza (*P. cinerascens*).

2.2. Objetivos específicos

1) Relacionar o teor de taninos dos frutos de cada espécie de planta com o peso das partes consumidas e descartadas dos frutos consumidos por *P. cinerascens*;

2) Relacionar o teor de alcaloides dos frutos de cada espécie de planta com o peso das partes dos frutos consumidas e descartadas por *P. cinerascens*.

Capítulo 1

Mattos FS, Koolen HHF, Pohlit AM, Negreiros AA & Barnett AA.
Influence of tannins and alkaloids on the choice of fruits eaten by
Plecturocebus cinerascens (Platyrrhini: Pitheciidae). Manuscrito
submetido para a revista American Journal of Primatology.

Influence of tannins and alkaloids on the choice of fruits eaten by

Plecturocebus cinerascens **(Platyrrhini: Pitheciidae).**

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ABSTRACT

Plants protect themselves against herbivores in many mechanical and chemical defense mechanisms. Much of the secondary metabolites that plants produce, e.g. tannins and alkaloids, have been shown to deter herbivores. It is possible that the amount of ingested mass of fruits consumed by *Plecturocebus cinerascens* (Platyrrhini: Pitheciidae) is lower depending on the concentration and type of secondary metabolites (tannins and alkaloids) contained in the fruits. The fieldwork was carried out in the area of the Rondon II Hydroelectric Power Plant, Pimenta Bueno, Rondônia State, Brazil. In order to record the species of fruit consumed by *P. cinerascens*, we searched the animals at seven different sites, each site being the living area of a distinct Ashy titi monkey group. Once we registered the feed of a certain group of Ashy titis, we were searching for a different group in order to avoid sample pseudo-repetition. We collected fruit samples to perform chemical analysis in all plants in the fruiting stage where the animals were fed. The chemical analysis was performed both in the consumed parts and in the unconsumed parts of the fruits. The results of the multiple regression did not show a relation between the concentration of secondary metabolites and the weight of the freshy mass of the fruits consumed by Ashy titi monkeys. However, the animals discarded the parts of the fruits in the only plant species (*Amorimia* [*Mascagnia*] *rigida* [Malpighiaceae] and *Inga* sp. [Fabaceae: Mimosoideae]) that presented alkaloids. We conclude that the diet of *P. cinerascens* is not influenced by secondary metabolites (e.g. tannins and alkaloids) and indicates a broader investigation of the chemical composition of food items that *P. cinerascens* consumes to evaluate if other chemical compounds, not evaluated in this study (e.g. proteins, lipids, sugars and others), influence the diet of these primates.

Key works: Ashy titi monkey, plant secondary metabolites, primates, Malpighiaceae, phytochemicals, fruit consumed.

INTRODUCTION

Free-ranging primates must deal with a number of external factors that can influence the composition of their diet. These include, competition for existing resources in a given area [Dew, 2005; Ganzhorn, 1988; Palminteri et al., 2013], temporal and spatial availability of food needed to meet daily energy requirements [Heiduck., 1997; Mourthé, 2012; Stevenson et al., 2000; Souza-Alves et al., 2011; Stevenson, 2001; Worman & Chapman, 2006; Wrangham et al., 1998], nutritional quality of available items [Glander, 1982; Guo et al., 2007; Milton, 1981], and the presence of defensive chemicals in the plants on which they might feed [Coley et al., 1985; Schmitz & Ritchie, 1991]. Solutions for all must be found and balanced if an animal is to successfully inhabit a particular environment [Wright, 1989].

Of the above, the chemical protection of plants via toxic substances (secondary metabolites) [Coley et al., 1985] has been the subject of a variety of studies due to their effects on animal time budgets [Freeland & Janzen, 1974; Schmitz & Ritchie, 1991], ability to reduce forage quality [Freeland & Janzen, 1974; Glander, 1982; Hartmann, 1991; Lev-Yadun et al., 2009; McKey et al., 1981; Oates et al, 1980], capacity to trigger intestinal disorders [Freeland et al., 1985] and even kill animals [Becker et al., 2013; Bezerra et al., 2012; Duarte et al., 2013; Freeland & Janzen, 1974; Lee et al., 2014; Peixoto et al., 2010]. For primates, while some studies report that investigated secondary metabolites have no influence on food choice [Chapman & Chapman, 2002; Li et al., 2015; Wrangham et al., 1998], others highlight not only their influence [Emerson & Brown, 2015; Kinzey & Norconk, 1993; McKey et al., 1981], but the adoption of strategies to prevent the ingestion of such toxins [Corlett & Lucas, 1990].

Among the four families of neotropical primates (Aotidae, Atelidae, Cebidae and Pitheciidae), the pitheciids (*Callicebus, Cheracebus* and *Plecturocebus* in the sub-family Callibinae, and *Cacajao, Chiropotes* and *Pithecia* in the Pitheciinae) are known as seed predators [Norconk, 2013]. In general, pitheciids do not ingest the whole fruit, but process them to access and consume very specific parts (seed, aril, pulp) [Ayres, 1986; Barnett, 2010; Boubli, 1999; Bowler, 2007; Norconk et al., 2009; van Roosmalen et al., 1988; Veiga, 2006]. The handling process is the result of development of morphological characteristics and skills that give these primates the capacity to exploit such food resources as hard fruits, which are little-used by other families of primates [Ayres 1986; Barnett, 2005; Boubli, 1999; Boyle, 2008; Kinzey & Norconk, 1990; Norconk, 2011; Norconk, 2013; Norconk & Conklin-Brittain, 2004; Norconk et al., 2009; Palminteri et al., 2013; Veiga, 2006].

The Ashy Titi (*Plecturocebus cinerascens*) [sensu Byrne et al., 2015] is one of the least-known Callicebids. Apart from appearance in taxonomic treatments, such as Auricchio [2010], Kobayashi, [1995], the species has not received detailed ecological studies as have other Callicebids (e.g. *Callicebus moloch* [Moynihan, 1966], *Callicebus coimbrai* [Souza-Alves et al., 2011], *Callicebus melanochir* [Heiduck, 1997]). Instead, prior to this study, the only field data recorded until now are about the species distribution and habitat preferences [Ferrari et al., 2000; Noronha et al., 2007]. Nothing has been published on diet or foraging ecology.

However, the dental and cranial morphology of *P. cinerascens* is typical for the genus [Kobayashi, 1995; Norconk et al., 2009], so that, based on diet studies of other titis [Alvarez & Heymann, 2012; Bicca-Marques & Heymann, 2013; Heiduck, 1997; Souza-Alves et al., 2011], it is expected that the species consumes fewer hard fruits that do the larger pitheciins. However, even they do not require the extremes of

investment in time and effort which the process that hard-husked fruits requires [Barnett et al., 2016] the items eaten by titis [Bicca-Marques & Heymann, 2013] requires a certain amount of selectivity, especially as they tend to consume small amounts of mass and discard the seeds, even though these are richer in nutrients and lipids [Norconk & Conklin-Brittain, 2004]. For some fruits (e.g. *Bellucia grossularioides*, Melastomataceae), *P. cinerascens* consumes about half fruit and discards the rest, seeking other intact fruit soon after to eat [Mattos, unpublished data]. As the theory of optimal foraging [MacArthur & Pianka, 1966], suggests animals should minimize energy expenditure when seeking food [Altmann, 2006; Garber, 1987; Kurland & Beckerman, 1985; Palminteri et al., 2016; Stacey, 1986], how can such apparent wastefulness, in both energetic and temporal terms, be explained? The influence of the chemical composition of the diet items may provide a possible explanation.

Therefore, in this study we tested the hypothesis that the consume of fruits in *P. cinerascens* is influenced by the presence and concentration of secondary metabolites (tannins and alkaloids) of the fruits, wherein: in terms of mass consumed, ingestion of fruit parts by Ashy titis will be direct inverse proportion to the concentration of tannins and alkaloids within them. So, our aim was to evaluate the influence of secondary metabolites (tannins and alkaloids) of fruits on consume of fruits of the Ashy titi (*P. cinerascens*).

MATERIALS AND METHODS

Study area

The study was conducted in forests surrounding the Hydroelectric Power Plant (HPP) Rondon II, located in the municipality of Pimenta Bueno, Rondônia (Fig. 1). The area is located 590 km from the state capital, Porto Velho. The HPP Rondon II has a total area of 16,000 hectares of legally preserved forest, which protects both watershed and wildlife. The vegetation is characterized as transition zone between Savannah and Open Ombrophilous Forest and between Savanna and Seasonal Evergreen Forest [RADAMBRASIL, 1978; IBGE, 2012]. Near the Apertado da Hora Canyon of the Rio Comemoração, rocky outcrops occur where there is low and sparse vegetation. In some of these places the soil is shallow and sandy, flooding in the rainy season. The soil of the region is classified as Quartzipsamments hydromorphic with sandy texture and low relief [IBGE, 2006]. The Köppen classification of the local climate is Aw, being warm moist tropical with winter dry season [IBGE, 2002; Kottek et al., 2006]. The dry and rainy seasons last an average of three and nine months, respectively [IBGE, 2002].
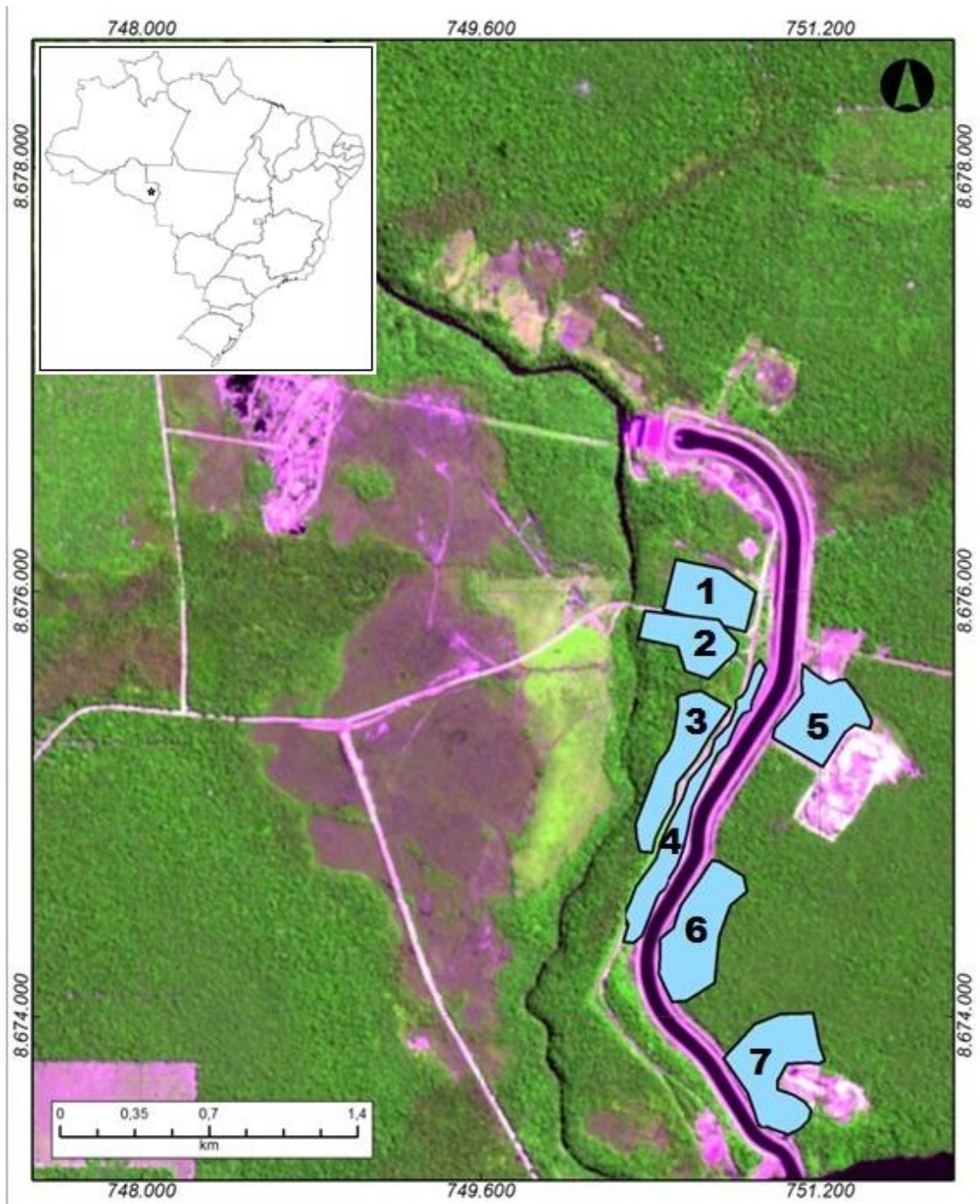
Figure 1. Satellite image of the Rondon II Hydroelectric Power Plant, Pimenta Bueno, Rondônia State, Brazil. The polygons show forest fragments where data and botanical collections were made.

Study species

The Ashy titi, *P. cinerascens* (Fig. 2), belongs to the subfamily Callicebinae (family Pitheciidae), the most species-rich taxon within the order Primates. The members of this taxon are divided into four different groups, and *P. cinerascens* is indicated as the more oldest ancestral form of the "moloch-group" [Byrne et al., 2015]. Average weight is 1 kg [Ford & Davis, 1992], head-&-body measures 33-38 cm, with a tail of about 45 cm [Hershkovitz, 1990]. Groups of up to six are formed, with solitary individuals also seen [Mattos, unpublished data]. However, most frequently groups of four individuals are seen; couple of adults and two younger individuals (the couple's children), which is the commonest group size for titis [Souza-Alves et al., 2011]. Though, there is no quantitative data on *P. cinerascens*, the skull and dentition possess no unusual modifications (Kobayashi 1995) and so the species' diet is expected to be the mix of fruits (whole or part), buds, flowers and arthropods (spiders, termites and ants), typically eaten by other titis [Rosenberger, 1992]. Individual *P. cinerascens* manipulate fruits to feed on specific fruit parts such as seeds, pulp or aril [Mattos, unpublished data]. Like other titis [Moynihan, 1966], *P. cinerascens* emits a range of vocalizations including calls for danger and contact, as well as early morning and later afternoon calls to demarcate territory [Mattos, personal observation].

Figure 2. Adult female of *Plecturocebus cinerascens* (Platyrrhini: Pitheciidae). Photo: Mattos, FS.

Data Colletion

Feeding data collection

Data collection occurred during monthly field visits of 9 days per month, between May 2015 and April 2016. During this time data was collected on *P. cinerascens* feeding, along with associated botanical information. Titi groups were found by during transect walks, either visually or by their vocalizations. Transects used pre-existing trails and rural roads at the edges of forest fragments. The location of each plant on which the animals were seen foraging had geographic coordinates recorded by GPS device (Garmin Etrex 30), then labeled with autoclave tape, to facilitate subsequent access to botanical collections. Seven sites in the region were sampled, to maximize data-sampling and avoid sample pseudo-repetition [Magnusson & Mourão, 2005]. Due to lack of preexisting information on *P. cinerascens*, study sites were separated by a distance approximating to the home range of *Callicebus coimbrai* studied by Souza-Alves et al. [2011], which apparently

has the same home range [Mattos, personal observation]. Records of feeding were obtained using a method adapted from scan sampling [Altmann, 1974], with binoculars (10x42 Barr & Stroud Skyline), because we only needed record the plant species and what fruit part the animals were feeding. For this reason, the animals were scanned during all the time that we could see them in the field.

Botanical data collection

Specimens were collected from individual plants with leaves branches and fruits and / or flowers where *P. cinerascens* was seen feeding. Collections followed the standard protocol of the Queensland Herbarium [2013], and all material collected was identified with the help of floras [e.g. Pennington, 1990; Ribeiro et al., 1999; van Roosmalen 1985] and experts (see acknowledgments). Along with the material collected for identification, fruits rejected or partially-eaten by titis during feeding were collected from feeding trees. Both these, fruits partially-eaten and intact fruits were used in the chemical analyzes (see below) to calculate the average of weight freshy mass of each consumed species. To calculate the average of weight freshy mass of the fruits from a particular species we weigh all fruits collected (Table 2), sum their values and then we divide the result of the sum by the number of total fruits weigh. All fruit destined for chemical analysis were stored in species-specific packages in a refrigerator at a constant temperature (-12°C) to prevent chemical degradation and to halt the ripening process [Atlabachew et al., 2013; Rogez et al., 2004]. For transport to the analytical lab fruit samples were placed in coolers containing dry ice and transported by air to Manaus (1,100 km).

Chemical analysis

Chemical analyzes were performed in the Amazonas State University (UEA) in Bioorganic Laboratory of the Master's Program in Biotechnology and Natural Resources. Alkaloid content of samples (Table 1) was assayed using the alkaline precipitation method of Harborne [1973]. Material was divided into eaten and non-eaten parts of the diet item (e.g. sarcotesta [eaten], seed [not-eaten] of *Inga*), and triplicates analysis was performed to provide an average of alkaloid concentration [Manikpuri & Jain, 2015]. This analysis is semi-quantitative and is performed putting 2g of fruit material on an Erlenmeyer recipient. Then, we added 80ml of mix 20% acetic acid in methanol and leave to stand for 4 hours. After this, the solution was filtered and, after filtering, we concentrated the solution by evaporating 90% of the solvent previously added. After that, the solution was filtered and, after filtering, we concentrated the solution by evaporation 90% of the solvent previously added. Then, the concentrated solution was put on Becker recipient where we added 1ml of drops of ammonium hydroxide until the formation of some precipitated material. If it is not occurs, we discarded the assay. Nonetheless, if the precipitation occurs, we filtered the solution with a thin paper and weigh the material. The weight resulting give us the quantity of alkaloid.

To assays of tannin concentration (Table 1) we use the modified method of the acidified vanillin [Burns, 1971], which allows a rapid assessment of the relative content of tannins. First, we prepared a solution with equal volumes of 8% of concentrated hydrochloric acid (HCl) in methanol and 4% of vanillin in methanol just before use. Then, we prepared a standard curve by adding 100 mg catechin to 50 ml methanol. We use at various dilutions to construct standard curve. We did it pipetting 1ml of each dilution into each of two separate tubes. After the 10 dilutions (20 tubes)

have been prepared, we quickly added 5 ml of vanillin-HCl reagent to each. After, we read on spectrophotometer at 500 mμ after 20 minutes. The vanillin-HCl was used for the 100% transmittance blank. So, we plotted the transmittance against catechin concentration. For the determination, we put 1 g of ground fruit material in flask and added 50 ml methanol to each flask, stoppered and swirled. We mix occasionally by swirling. After 28 hours swirl and let settle. Then, we pipetted 1 ml of supernatant into each of two tubes and proceeded as with description above. Finally, we extract at one time all samples and compared them.

Data analysis

We first test the normality of the data with Shapiro-Wilk test. The result shows that the data had no normal distribution. Then, we applied the Spearman non-parametric test to evaluate correlation between variables. The cutoff value for considering the correlated variables was Rho=0.60 [Zuur et al., 2009]. The result of the Spearman test not showed correlation between weight of freshy mass of eaten and not-eaten fruit parts (t = 0.1204, df = 8, P = 0.9071) and between concentration of tannins of eaten and not-eaten fruit parts (t = -0.7697, df = 11, P = 0.4577). We did not perform test for alkaloid because there were many zeros for consumed and unconsumed fruit parts. After these procedures, we applied a generalized linear model (GLM) with quasipoisson dispersion parameter (to correct standard errors) because an overdispersion was detected on the set of variables [Zuur et al., 2009]. So, data analysis was performed by multiple linear regression [Gotelli & Ellison, 2011] in software R 3.2.1 [R Core Team, 2013], using as predictor variables: 1) the concentration of tannins, and 2) the concentration of alkaloids in consumed parts and discarded parts of the fruits of each species of plant recorded as eaten by *P.*

cinerascens. The sampling unit was the plant species and we use as response variable the weight of freshy mass expressed in grams. To standardize values for the statistical treatment, average weights of fruits (or their parts) were calculated as means (Table 2), and plant species where no secondary metabolites were detected, were given the value zero.

RESULTS

Over the 12-month study period, a total of 408km of trails were walked and 700 hours looking for titis was performed over 100-field days. During this time, 65 feeding records were made of 07 different *P. cinerascens* groups (07 different locations each one) within the study site. Data collected included 56 feeding records in fruits. These came from 12 different plant species.

***Chemical composition of the plants eaten by* P. cinerascens**

Alkaloid and tannin levels were obtained for the 12 plant species *P. cinerascens* was recorded eating during the study (Table 1). These came from 11 botanical families (Table 2). Tannins were recorded for all species, while alkaloids compounds were recorded in fruits of only two species of plants: *Amorimia* [*Mascagnia*] *rigida* (Malpighiaceae) and *Inga* sp. (Fabaceae: Mimosoideae).

The values in Table 1 refer to the mean and standard deviation of the chemical concentration of tannins and alkaloids from consumed and discarded parts of the fruits eaten by *P. cinerascens* of each species of plant. For tannins, the values refer to milligram (mg) of catechin equivalent/100g of fresh fruit and, for alkaloids, the values refer to mg/g of fresh fruit.

Effects of tannins and alkaloids on consume of fruits by P. cinerascens

The multiple regression analysis (Fig. 3) not showed the existence of a relationship (F = 0.6416; p = 0.4495) between the freshy mass and the concentration of tannins in the parts of the fruits that were consumed by the titis. The results for alkaloids also did not show a relationship between the freshy mass and the concentration of alkaloids in the consumed parts of fruits.

Table 1. Concentration of plant secondary metabolites (total tannins and alkaloids) of fruits of plant species consumed by *P. cinerascens* (Platyrrhini: Primates) in the area of Rondon II Hydroelectric Power Plant, Pimenta Bueno, Rondônia State, Brazil. ND = Not detected in the analysis.

Consumed plants	Consumed part	Not consumed part	Tannin concentration of fruits (mg / 100g)		Alkaloid concentration of fruits (mg / g)	
			Consumed part	Not consumed part	Consumed part	Not consumed part
Passiflora coccinea	seeds	seeds and peel	4.55 ± 0.65	4.14 ± 0.62	N.D.	N.D.
Bellucia grossularioides	half fruit	half fruit	74.11 ± 8.76	74.11 ± 8.76	N.D.	N.D.
Hirtella racemosa	whole fruit	---	3.45 ± 0.57	---	N.D.	N.D.
Inga alba	testa	seeds	3.55 ± 0.58	3.67 ± 0.50	N.D.	N.D.
Matayba guianensis	testa	seeds	19.12 ± 3.12	3.15 ± 0.53	N.D.	N.D.
Mouriri acutiflora	aril	seeds	84.20 ± 9.21	3.22 ± 0.51	N.D.	N.D.
Amorimia Mascagnia rigida	seeds	winged part	4.16 ± 0.67	23.72 ± 4.18	N.D.	1.02 ± 0.15
Inga sp.	testa	seeds	12.65 ± 2.71	3.83 ± 0.50	N.D.	1.51 ± 0.21
Pouteria ramiflora	testa	seeds	9.28 ± 1.88	4.03 ± 0.60	N.D.	N.D.
Protium heptaphyllum	testa	seeds	6.61 ± 1.25	4.49 ± 0.90	N.D.	N.D.
Qualea paraensis	seeds	winged part	6.13 ± 0.76	5.81 ± 0.62	N.D.	N.D.
Pourouma bicolor	testa	seeds	5.02 ± 0.73	4.19 ± 0.64	N.D.	N.D.

Table 2. Average weight of fruit parts consumed and not consumed of each plant species by *P. cinerascens* in the area of the Rondon II Hydroelectric Power Plant, Pimenta Bueno, Rondônia State, Brazil. ND = Not detected in the analysis.

Consumed plants	Botanic family	Nº of collected fruits	Average of weight of consumed part (g)	Average of weight of not consumed part (g)
Passiflora coccinea	Passifloraceae	20	12.580	12.580
Bellucia grossularioides	Melastomataceae	20	7.6500	7.6500
Hirtella racemosa	Chrysobalanaceae	20	0.0165	0.0165
Inga alba	Fabaceae	40	0.0190	2.6400
Matayba guianensis	Sapindaceae	100	0.0010	0.0054
Mouriri acutiflora	Memecylaceae	50	7.2700	ND
Amorimia Mascagnia rigida	Malpighiaceae	20	0.0150	0.0160
Inga sp.*	Fabaceae	---	ND	ND
Pouteria ramiflora	Sapotaceae	11	0.1850	3.3100
Protium heptaphyllum	Burseraceae	06	ND	0.2983
Qualea paraensis	Vochysiaceae	07	0.0300	ND
Porouma bicolor	Cecropiaceae	04	ND	0.1800

* All material was used for chemical analysis.

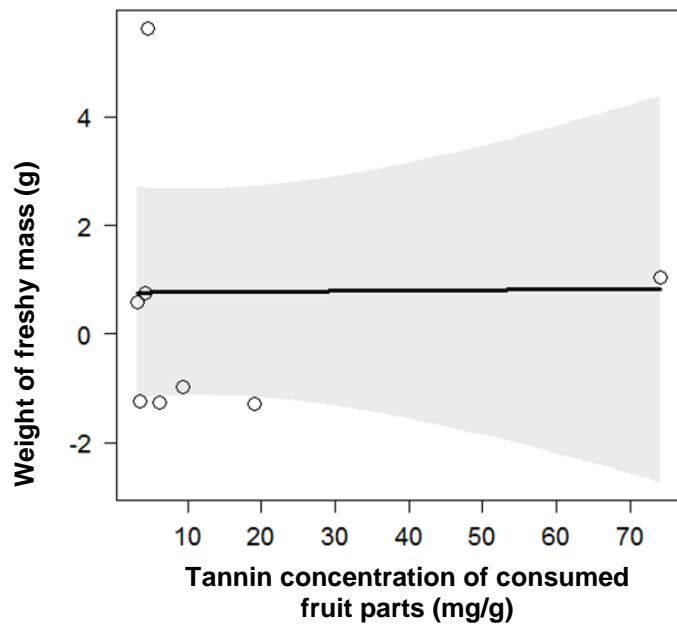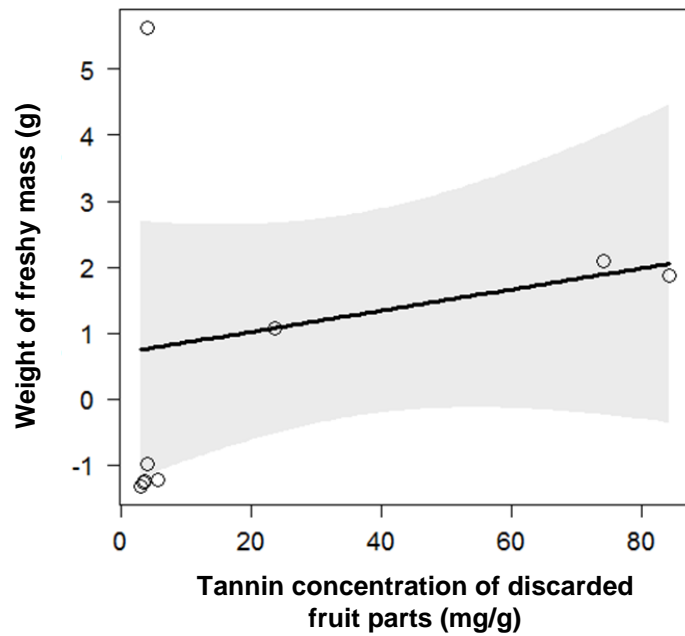**Figure 3. Relationship between tannin concentration and weight of freshy mass of the fruits eaten by *P. cinerascens*. Points on the graphs are species of plants. The first graphic shows the relationship between weight of freshy mass of fruits and the tannin concentration of discarded fruit parts and the second graphic shows the relationship between weight of freshy mass of fruits and tannin concentration of consumed fruit parts.**

DISCUSSION

***Production of taninns and alkaloids in fruits eaten by** P. cinerascens*

Analysis revealed that all 12 plant species on which *P. cinerascens* was recorded as feeding invest in the production of tannins, while alkaloid compounds were present in two species (*Amorimia* [*Mascagnia*] *rigida* and *Inga* sp.). Secondary metabolites, such as tannins and alkaloid, produced by plants are widely considered to act in defense against herbivores [Wink, 2003].

In studies, such as tannins and alkaloids are widely produced in plants [Edeoga et al., 2005; Jung et al., 1979], and are the most frequent secondary metabolites Jung et al. [1979]: only one of the 10 species listed by Edeoga et al. [2005] lacked tannins. However, the presence of alkaloids was higher than that of tannins in plants studied [Jung et al., 1979].

However, in the current study, alkaloids were found in only two plant species. Reasons for the departure from a near-global pattern may lie with the local soil characteristic, as water stress and nutrient-availability, and light intensity can affect the production of secondary metabolites [Gershenzon 1983; Waterman et al., 1984: see Vrieling & van-Wijk, 1994 for a telling example with *Senecio jacobaea,* Asteraceae]. Soils of the southern-most part of the Amazon Basin wherein HPP Rondon II is located are notably poor [IBGE, 2006], and the dry season is protracted [IBGE, 2002]. Thus it is possible that local aspects of soil and climate are involved, but this should be the subject of a specific future study.

Agreement between results and hypotheses

Hypothesis: in terms of mass consumed, ingestion of fruit parts by Ashy titis will be direct inverse proportion to the concentration of tannins and alkaloids within them

Although condensed tannins may combine with proteins, rendering them unavailable or digestible [Milton, 1984; Rothman et al., 2006], the current study did not find such a relationship between the concentration of tannins and the mass of fruits parts consumed by *P. cinerascens*. This because in some analysed plants, such as *Mouriri acutiflora* (Memecylaceae), *Matayba guianensis* (Sapindaceae) and *Pouteria ramiflora* (Sapotaceae), the concentration of tannins in the parts that animals consumed are much higher than those parts that were discarded (Table 1). A study by Heiduck [1997] of an Atlantic forest titi (*Callicebus melanochir*) also found that phyto-chemicals have no apparent influence on food selection. So it may be that other factors influence the fruit consumption patterns in this group.

One of these may be the maturation stage of the eaten fruit. Of the 12 fruit recorded here, all were consumed at mature stage. Fruit at this stage are generally more digestible [Ganzhorn et al., 2009; Wink, 2003] and has more protein [Chapman & Chapman, 2002], as immature are often richer in secondary metabolites [Cipollini & Levey, 1997; Rhoades & Cates, 1976; Wink, 2003]. Thus, it is possible that the fruits consumed by *P. cinerascens* have higher concentrations of tannins when young, and when mature, have a low enough concentration to make them suitable for consumption. A situation similar to that described for the Grey-cheeked mangabey (*Lophocebus albigena*), where the animals consumed seeds and arils at the ontogenic stages that had the lowest concentrations of tannins [Masette et al., 2014].

Also related to the ontogenetic state, Chapman & Chapman [2002] studied *Procolobus badius* groups and reported that secondary metabolites have no influence on feeding these animals, who select younger leaves because of their higher digestibility. Likewise, Carlson et al. [2013] reported that chimpanzees consume leaves of some plant species in periods of the day when the quality of these resources is higher with more non-structural carbohydrate, for example.

The lack of relation between the intake of food items and secondary products has been suggested in animals that mutualistic associations with bacteria capable of degrading chemical molecules of secondary metabolites [McKey et al., 1981; Milton, 1984; Oates et al., 1980]. Since plant secondary metabolites may either pre-ingestion (as anti-feedants) or post-ingestion (reducing food digestability) [Rhoades & Cates 1976] it has been suggested that herbivorous animals may have acquired tolerance to plant chemical defenses [Rhoades, 1985], and this may envolve the composition and capacities of the intestinal flora [Taguer & Maurice, 2016]. In addition, some primates are known to use produce small tannin-attractive proteins in their saliva which effectively disable tannins by combining with them before they reach the gastro-intestinal canal (Gómez et al., 2016).

Overall, pattern-seeking generalization may be difficult since, related species in different regions may exhibit varying degrees of tolerance to secondary metabolites [Calvert 1985], and different lineages may respond very differently to the same suite of circumstances. For example, while chimpanzees prefer ripe fruit and actively avoid foods rich in dietary inhibitors, guenons (*Cercopithecus* spp.) in the same forests ingest foods with large amounts of secondary metabolites [Wrangham et al., 1998].

Comparing mountain and western lowland gorillas, Calvert [1985] reported that the former were less of tolerant tannins than the latter, and that the lowland gorillas area had poorer spoils, where tannin levels would be expected to be higher. This suggests adapation by primates to the chemical profile of the plants in their region and, consequently, diet. Relativistic aspects may also operate during within-site food item selection: for example, choice tests with fallow deer Bergvall & Leimar [2005] found that the deer consumed more trays of food with lower tannin concentrations. However, the animals only showed selectivity when the difference in the concentration of tannins was pronounced. When the diferences were not extensive, the deer ceased to choose between the feed-containing trays [Leimar & Bergvall, 2005].

However, an analysis of the factors influencing fruit removal of all avian frugivores (84 species) interacting with 33 tree species in a Southern Veneuelan forest, Schaefer et al. [2003] found than the decrease in the concentration of phenols (and not tannins or alkaloids) seemed most closely linked to the onset of fruit removal.This opens the possibility that other types of secondary metabolites, other than those evaluated in this study, may have had an influence on titi forage choice.

Alkaloids were recorded only for two species of plant. In both cases they were present in parts of the fruit rejected (the winged part of *Amorimia* [*Mascagnia*] *rigida* and the seeds of *Inga* sp.) by Ashy titis during their feeding. This apparent avoidance allies with that has been reported in other species: alkaloids are well-known for their toxic effects [Rhoades & Cates 1976], and were listed as the main factors in species and part avoidance by *Papio anubis* [Barton & Whiten, 1994], while for *Colobus satanas* leaf-choice is strongly influenced by alkaloid levels [McKey et al., 1981]. Detoxifying alkaloids is energetically costly and Marsh et al., [2006] have suggest

that primates adjust the composition of their diet so as moderate the energetic investment in this function.

The avoidance of alkaloid-containing fruit parts by *P. cinerescens*, may be highly adaptive since parts of the fruits one of the species involved, *A. [M.] rigida*, appear to be lethally toxic. The winged part of fruits in the genus *Amorimia* contain sodium monofluoracetate, a substance documented as responsible for the death of cattle, sheep, goats and rabbits [Becker et al., 2013; Duarte et al., 2013; Lee et al., 2014; Peixoto et al., 2010]. Monofluoracetate becomes toxic after undergoing intracellular molecular changes which result in blocking the cell respiration [Tokarnia et al., 2002].

Mammals may learn from their mothers to avoid *Amorimia* [Becker et al., 2013] Learning about the food is by transferring the food preferences of the mother, as is the case with other food preferences [Watts, 1985; Galef & Giraldeau, 2001]. Such mechanisms are known in primates [Hikami et al., 1990; Visalberghi & Addessi , 2000; van de Waal et al., 2013], making it possible that individual *P. cinerascens* are transferring to younger individuals of the fruit handling behaviors they use to avoid ingestion of lethal toxins.

Conclusions

Based on the results of this study, we conclude that tannins and alkaloids have no influence on titis feeding. However, further studies on alkaloid effect should be made to clearly define whether this group of plant secondary metabolites has influence on the *P. cinerascens* feeding, or whether they are feeding in a period that the fruits are qualitatively better with less concentrations of alkaloids.

Fruit choice clearly involves a multiplicity of factors. Consequently, we suggest new studies that investigate whether concentrations of plant secondary metabolites change seasonally and/or ontogenically, as well as investigations of the links between soil quality and plant secondary metabolites would be of great value, as would a flora-wide assay of phytochemical production in the Rondon II Hydroelectric Plant area. In addition, we recommend carrying out studies on the ability of both titi intestinal flora and saliva in nullifying tannins in diet items. Finally, we suggest studies that aim to test whether environmental factors such as the diversity of fruits and/or fruiting peaks exert effect on *P. cinerascens* feeding.

ACKNOWLEDGMENTS

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3. Conclusão

Com base nos resultados desse trabalho, concluímos que os metabólitos secundários, especialmente taninos e alcaloides, não exercem influência no consumo de frutos pelos zogue-zogues-cinza. Contudo, um estudo mais amplo sobre o efeito de alcaloides deve ser feito para definir claramente se este grupo de metabólitos secundários possui alguma influência na alimentação de *P. cinerascens* ou se eles estão se alimentando em um período que os frutos estão qualitativamente melhores.

Ademais, sugerimos novos estudos que se concentrem em avaliar se existe variação na concentração de metabólitos secundários das plantas ao longo das estações do ano para verificar se a ocorrência de alcaloides está relacionada à estresses ambientais ou se a produção destas substâncias é realizada por somente poucas espécies de plantas da área da UHE Rondon II. Além disso, indicamos a realização de estudos sobre a capacidade da flora intestinal dos zogue-zogues em anular o efeito de taninos na alimentação destes animais. Por fim, sugerimos a realização de estudos que objetivem testar se fatores ambientais, como a diversidade de frutos e/ou picos de frutificação, exercem efeito sobre a alimentação do *P. cinerascens*.

4. Ata de defesa

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA

ATA DA DEFESA PÚBLICA DA DISSERTAÇÃO DE MESTRADO DO PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA.

Aos 30 dias do mês de agosto do ano de 2016, às 14h00, no Auditório do LBA, Campus II, INPA/ALEIXO reuniu-se a Comissão Examinadora de Defesa Pública, composta pelos seguintes membros: o(a) Prof(a) Dr(a) **Igor Luis Kaefer** , da Universidade Federal do Amazonas - UFAM, o(a) Prof(a) Dr(a) **Paulo Estefano Dineli Bobrowiec,** do Instituto Nacional de Pesquisas da Amazônia - INPA, e o(a) Prof(a) Dr(a) **Sérgio Massayoshi Nunomura,** do Instituto Nacional de Pesquisas da Amazônia - INPA, tendo como suplentes o (a) Prof(a) Dr(a) Fabricio Beggiato Baccaro, da Universidade Federal do Amazonas - UFAM, e o(a) Prof(a) Dr(a) Mario Cohn-Haft, do Instituto Nacional de Pesquisas da Amazônia - INPA, sob a presidência do(a) primeiro(a), a fim de proceder a argüição pública do trabalho de **DISSERTAÇÃO DE MESTRADO** de **FÁBIO DE SOUZA MATTOS**, intitulado: **"Dieta do *Plecturocebus cinerascens* (Platyrrhini: Primates): a influência da composição química na escolha de frutos consumidos"**. Orientado pelo(a) Prof(a) Dr(a) Adrian Ashton Barnett, do Instituto Nacional de Pesquisas da Amazônia – INPA e coorientada pelo(a) Prof(a) Dr(a) Valdir Florêncio da Veiga Junior, da Universidade Federal do Amazonas – UFAM.

Após a exposição, o (a) discente foi arguido(a) oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

[X] APROVADO(A)   [ ] REPROVADO(A)

[ ] POR UNANIMIDADE   [V] POR MAIORIA

Nada mais havendo, foi lavrado a presente ata que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Prof(a) Dr(a) IGOR LUIS KAEFER _____

Prof(a) Dr(a) PAULO ESTEFANO DINELI BOBROWIEC _____

Prof(a) Dr(a) SÉRGIO MASSAYOSHI NUNOMURA _____

Prof(a) Dr(a) FABRICIO BEGGIATO BACCARO _____

Prof(a) Dr(a) MARIO COHN-HAFT _____

_____
Dra. Rosalee Albuquerque Coelho Netto
Coordenadora da Pós-Graduação
PO 163/2015 MCTI/INPA