Using micro-histology and an image-based identification tool to study the diet of *Iguana iguana* (Linnaeus, 1758)

The adult Green Iguana, *Iguana iguana* (Linnaeus, 1758), is a Neotropical arboreal herbivore according to studies based on stomach contents, fecal samples and direct observations (Iverson 1982; Rand et al. 1990). This species represents one out of about 50 truly herbivorous lizard species worldwide (Mackie et al 2004) and rarely eats animal material such as tree snails, bird eggs, chicks and small mammals (Townsend et al. 2005; López-Torres et al. 2012). *Iguana* is an ectothermic hindgut fermenter using gut microbes to digest food (Mackie et al. 2004). In that Green Iguanas eat seeds, they play an important role in the reproductive strategies of both native and non-native plants throughout their range (Benítez-Malvad et al. 2003; Guzmán 2007). To understand this ecological role of Green Iguanas, plants consumed by the species needed to be identified. Despite this lizard's wide distribution throughout Brazil, ongoing studies focus mainly on its reproduction (Ferreira et al. 2002; Campos 2004; Campos & Desbiez 2013) and most recently coprophagy behavior (Campos et al. 2010), but not diet.

The purpose of this study was to test methods for the identification of plant species consumed by free ranging Green Iguanas without killing the lizards. For this purpose, the technique of micro-histological image analysis was applied to determine the botanical composition of plant materials found in the stomachs and faecal samples. This technique is widely used to examine the diet of mammals (Santos et al. 2002; Desbiez et al. 2011) but was never adopted to Green Iguanas.

The present study was conducted in the Private Reserve of Natural Heritage (PRNP) - SESC Pantanal (center: 16°31.636'S / 56° 22.772'W), in the Barão de Melgaço subregion, Pantanal, Brazil. In 2008 and 2009, Green Iguanas were located during night surveys while sleeping on branches of trees and shrubs on the banks of the Cuiabá River through the use of a sealed-beam light con-

nected to a 12 volt battery. Iguanas were captured using a lasso tied to a bamboo stick or by hand, depending on the time of the day and their perch height in the branches. Since Green Iguanas, when disturbed, jump into the water to avoid being predated or captured, the capture procedure had to be completed before the dozing lizard fully "awakened". Captured individuals were immobilized by sealing their eyes and mouth with duct tape to minimize stress of capture. Each iguana captured was measured for snout-vent length, total length and body mass. Two techniques to collect food items were applied: stomach flushing and bag fecal deposit. During the stomach flushing a tube is gently inserted into the animal's stomach to rinse it with water, while the other method involves securing the captured animal in a cool dry place in a cloth bag until it defecated.

A total of 10 Green Iguanas were captured for this study. In four of them the stomach washing technique was applied, while from six lizards bag fecal deposits were obtained. Fecal matter was deposited between one and 42 hours after capture. Fecal samples and stomach content were kept in 70 % alcohol for future analysis in the lab.

While in the field, about 100 plant species were collected on or near the riverbanks where the iguanas were found and classified according to habit growth form (tree, shrub, herb and vine) and subjective abundance (high, medium and low) from visual observation. Plants were herborized and stored in a herbarium at Embrapa Pantanal for subsequent identification. Another portion of the same plants collected were kept in 70 % alcohol and used to produce micro-histological slides to create a reference collection for the identification of plant particles in the faeces and stomachs (Sparks & Malechek 1968).

The authors adopted the micro-histology technique associated with DELTADIET software logging taxonomically relevant leaf anatomic descriptors of Pantanal plants (Desbiez et al. 2010) to identify fragments of plants found on stomach and feces slides. Information from twenty-five plant species collected was entered into the DELTADIET program, which already contained characteristics of over 200 plant species, and thus represented an interactive identification key of plant fragments in feces and stomachs (Desbiez et al. 2010). Leaf epidermal anatomic descriptors were based on Alvarez et al. (2010) and Santos et al. (2010).

Two micro-histological slides were made from each fecal or stomach sample. These samples were homogenized separately in a blender and mounted to microscope slides using Hoyer's solution. Slides were then air-dried for two weeks before being analyzed. Using the DELTADIET Software (Desbiez et al. 2010), two persons independently analyzed the sample slides to identify plants ingested by the iguanas. Slides were analyzed by visual comparison of micro-anatomical charcterisics in the sample slide and the illustrations provided by the DELTADIET software. The DELTADIET interactive key (Desbiez et al. 2010) was created by observations made from the reference collection and a detailed list of characters and descriptors. The unique feature of this illustrated interactive key is that characters can be processed in any order. Characters that are not available in a particular epidermal fragment on a slide, or whose interpretation is not clear to the user, can be omitted.

The Green Iguanas of this study (eight adult females - snout-vent length 31.0 – 35.5 cm and two juveniles - snout-vent length 12.5 – 13.3 cm) had eaten 23 plant species from 13 families including trees, shrubs, herbs and vines (Table 1), with predominance of vines such as *Ipomoea rubens* and *Cissus erosa.*

Studies of the diet of Green Iguanas based on analyses of removed stomachs render euthanasia necessary (e.g., 122 individuals in Rand et al. 1990; Guzmán-Ramírez 2007; Govender et al. 2012). On the other hand, Rivas et al. (1996) recommended the method of non-flushing stomach rinsing for evaluation of plants consumed by large iguanids without injuring the animals. Although obtaining fecal and/or stomach samples reportedly induced little stress to animals, only few studies involving this technique in plant-eating iguanids are available (Lichtenbelt 1993; Morrison et al. 2007; Herilala & Mori 2012).

Table1: Plant species found in the stomachs and feces of juvenile and female individuals ($N = 10$) of *Iguana iguana* (LINNAEUS, 1758), from the Pantanal, Brazil. NI - Number of individuals containing this plant.

Tab. 1: Die festgestellten Pflanzenarten aus den Mägen und Faeces von Jungtieren und Weibchen ($N = 10$) von *Iguana iguana* (LINNAEUS, 1758) aus dem Pantanal, Brasilien. Wuchsformen (vine – Kletterpflanze, tree – Baum, shrub – Busch, herbaceous – krautig. NI - Anzahl der Individuen, welche die Pflanze beinhalteten.

Plant species Pflanzenart	Family Familie	Habit growth form Wuchsform	Abundance Häufigkeit	NI
Cissus spinosa	Vitaceae	vine	high / hoch	10
Panicum sp.	Poaceae	herbaceous	low / gering	1
Dicotyledons (not identified)	?	?	high / hoch	10
Ipomoea rubens	Convovulaceae	vine	high / hoch	10
Inga uruguensis	Mimosaceae	tree	high / hoch	6
Cecropia pachystachya	Cecropiaceae (Urticaceae)	tree	medium	8
Mikania micrantha	Asteraceae	vine	high / hoch	1
Grass (not identified)	Poaceae	herbaceous	medium	7
Doliocarpus dentatus	Dilleniaceae	vine	high / hoch	4
Alchornea castaneifolia	Euphorbiaceae	shrub	high / hoch	3
Cynodon dactylon	Poaceae	herbaceous	low / gering	2
Paullinia spicata	Sapindaceae	vine	medium	3
Malvaceae (not identiified)	Malvaceae	herbaceous	high / hoch	8
Banara arguta	Flacourtiaceae	tree	high / hoch	5
Triplaris gardneriana	Polygonaceae	tree	high / hoch	1
Sapium obovatum	Euphorbiaceae	shrub	low / gering	3
Murdannia sp.	Commelinaceae	herbaceous	medium	8
Eclipta prostrate .	Asteraceae	herbaceous	medium	4
Ipomoea carnea fistulosa	Vitaceae	shrub	high / hoch	8
Genipa americana	Rubiaceae	tree	high / hoch	4
Monocotyledons (not identified)		herbaceous	high / hoch	3
Arecaceae (not identified)	Asteraceae	-	low / gering	1
Mimosa sp.	Mimosaceae	shrub	low / gering	2

Only a small number of stomachs (4) and feces (6) were analyzed in this preliminary study, focussing on the optimization of methods to reveal the food composition of Green Iguanas in the wild. Plant species diversity was higher in the feces than stomachs. This difference was probably due to difficulties to implement stomach washing, which was found difficult to perform, contrary to what was reported in the study by RIVAS et al. (1996). Another reason may be the rapid passage of food through the stomach which stores food of about one day (RAND et al. 1990), compared to the intestinal food transit time of 2-8 days (LICHTENBELT 1993) which, due to the lizards' low metabolic rate, is prolongate relative to mammalian and avian herbivores.

Green Iguanas are considered opportunistic feeders (BURGHARDT & RAND 1982). Among the 23 plant species identified in the diet of *Iguana* from the Pantanal, 13 were visually classified as highly abundant, five were of medium and five of low abundance. Similar results were found by RAND et al. (1990) suggesting that the diet chiefly depends on the plants' availability (BURGHARDT &RAND 1982). The consumption of plants of low abundance also indicates selectivity. The animals consumed plants of different strata (tree, shrub, herbs and vines) and probably foraged continuously in the same place during the day since only few plant species were found in each sample. The presence of grass found in two Green Iguanas reveals that this arboreal species occasionally forages on the ground.

In summary, the use of fecal samples and micro-histologocal techniques turned out to be an efficient way to study the botanical composition of Green Iguana diet. This species is likely to play an important role as a seed disperser of plants consumed and, as a consequence, in the maintenance of riverbanks. However, Green Iguanas living alongside the Cuiabá River are threatened by changes in the flood pulse due to construction of hydroelectric dams which may flood their nests during the breeding season, erosion of the river banks due to cattle ranching, and the banks' indiscriminate use by fishermen. Green Iguanas should be the

object of further studies and used as ambassadors for conservation of riverbanks.

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