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Distinctive characteristics in adult female howler monkeys (*Alouatta palliata mexicana*) on the Agaltepec island, Veracruz, México

Características distintivas en hembras adultas de mono aullador de manto (*Alouatta palliata mexicana*) en la isla Agaltepec, Veracruz, México

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In primatology, the interest in research on females is growing for a better understanding of aspects like social interactions, maternal care, and female animal health. The correct identification of focal subjects is a major challenge in such studies. Adult female mantled howler monkeys (*Alouatta palliata*) illustrate well the methodological limitations. During a study on the Agaltepec island in Catemaco, Veracruz, México, we came across this challenge. As a strictly arboreal species howler monkeys are often located at higher levels covered by canopy which reveals only few parts for identification. Also, females with small offspring tend to stay further away from observers, even if fully habituated. In order to be able to begin with accurate data collection, a detailed identification of all adult females was completed. A total of 23 animals was described. The descriptions of the females refer mainly to their hands, feet and tail; the body parts that show signs of coloration. The necessity of the animal recognizability often causes young scientists to despair and leads to the loss of valuable investigation time. To facilitate the next generation of researchers on the Agaltepec island in their data acquisition, we present the detailed composition and description of the resident, adult females. We hope to provide reliable support for an efficient start of new projects.

Key words: Female animal health; field biology; field work; focal animal; focal collection; Mexican primates; primatology.

El interés por la investigación sobre las hembras ha crecido en primatología para comprender aspectos como las interacciones sociales, los cuidados maternos y la salud animal femenina. La correcta identificación de los sujetos focales es un reto importante en este tipo de estudios. Las hembras adultas de mono aullador (*Alouatta palliata*) ilustran bien las limitaciones metodológicas. Durante un estudio en la isla Agaltepec en Catemaco, Veracruz, México, nos encontramos con este reto. Al ser una especie estrictamente arborícola, los monos aulladores se encuentran a menudo en los niveles más altos, cubiertos por el dosel, por lo que revelan sólo algunas partes para su identificación. Además, las hembras con crías en edad infantil tienden a permanecer más alejadas de los observadores, incluso si están completamente habituadas. Para iniciar una colección de datos precisa, se completó una identificación detallada de todas las hembras adultas. Se describieron un total de 23 animales. Las descripciones de las hembras se refieren en particular a las manos, los pies y la cola, las partes del cuerpo que muestran signos de coloración. La necesidad de reconocer a los animales desanima frecuentemente a los jóvenes científicos y les hacen perder un valioso tiempo de investigación. Para facilitar a la próxima generación de investigadores de la isla Agaltepec la adquisición de datos, presentamos la composición y descripción detallada de las hembras adultas residentes. Esperamos proporcionar un apoyo fiable para el inicio eficaz de nuevos proyectos.

Palabras clave: Animal focal; biología de campo; colección focal; primates mexicanos; primatología; salud animal femenina; trabajo de campo.

Behavioral studies of free ranging animals often begin with a very similar challenge: habituating and identifying the subjects. Whereas, the habituation process requires patience and persistence (Williamson and Feistner 2003), individual identification further requires the ability to identify phenotypical differences between individuals, that are reliable and, preferably, consistent or less variable over time.

The identifiers differ for every species. For example, in zebras the individuals are discriminated by the different shapes of the coat markings (Lahiri *et al.* 2011), in humpback whales researchers look for slight morphological differences in shape of the dorsal fin (Katona *et al.* 1979), while bonobos are easiest distinguished by the shape and color of their genitals (pers. comm., A. Gisbrecht). Depending on the target species and the field conditions, programs like biometric databases or image retrieval systems can support accurate identification (Lahiri *et al.* 2011; Mendoza *et al.* 2011; Duyck *et al.* 2015). Unfortunately, the use of such programs is not always feasible.

Often, behavioral data is recorded by targeted observation of specific behaviors in defined settings such as a certain amount of time (Barker 1980). Examples for behavioral data are: general behavior of a group or sub-group (Pope 2000), juxtaposition of certain individuals (Calegaro-Marques and Bicca-Marques 1993; Kelaita *et al.* 2011), census of the group or identification of injured animals (Clarke *et al.* 1998). Collection of focal data enables an accurate statement about certain behaviors or group dynamics (Pope 2000; Dias *et al.* 2008). However, some of the animals might be shy and therefore harder to follow, which unfortunately could reflect the behavior and preferences of more confident individuals in the data.

The genus *Alouatta* is among the most studied primate taxa. The broad amount of knowledge about howler monkeys allows to move from the superficial questioning such as general diet or group composition (Asensio *et al.* 2007) and target the research more specifically, like subtle behaviors as the greeting ritual between males (Dias *et al.* 2008) or the role of secondary plant compounds in their diet. Further, in present-days the interest in female health and female specific behaviors is growing, which changes the fundamental research approach (Gao *et al.* 2015; Cano-Huertes *et al.* 2017; Hall and Klein 2017). The success of the studies on sociosexual or socioecological behaviors between members, female leaderships and reproductive investment of females highly depend on a correct identification of the individuals (Calegaro-Marques and Bicca-Marques 1993; Cano-Huertes *et al.* 2017; Pope 2000). However, the field research is mostly conducted by students with little field experience who are just at the beginning of their careers. Adequate identification attempts can cost valuable field time; in turn, time pressure can cause the field biologist to generalize patterns and misidentify the focal animals.

The Agaltepec island in Catemaco, Veracruz, México is inhabited by a group of mantled howler monkeys (*Alouatta palliata mexicana*). These animals were introduced as part of a translocation project in 1987 (Rodríguez-Luna *et al.* 1993) and are subject to studies since. The animals do not have any human interaction apart from the field researchers. In 1993, Rodríguez-Luna *et al.* described the majority of the individuals, but these descriptions hardly apply anymore. The average life span of mantled howler monkeys in the wild is 25 years with the highest ever recorded life span of a howler monkey accounting for 34 years (Glander 2006). This means that the group has changed in composition and the individuals who were residents in 1993 might no longer be present. Since our project focuses on females, we were able to create a detailed identification list of all resident adult females on the island.

Our aim is to support upcoming researchers on the Agaltepec island for an efficient approach to their projects. Further, to avoid delays of data collection and discrepancies in the data we provide guidelines for new identification and re-identification to facilitate targeted data collection.

The identification list was generated during a research stay between September 2021 and August 2023. The associated study focused on physiological requirements of female howler monkeys during different reproductive stages (cycling, gestating, lactating). A group of fully habituated mantled howler monkeys (*Alouatta palliata mexicana*) with a total of 23 adult females was monitored for a period of 24 months. The project focused exclusively on females; for that reason, an accurate count of males and subadults was not conducted. Of the estimate according to, the group consists of 13 males and 9 subadults in addition to the females. However, these numbers are approximations based on a few censuses of the group. Exact numbers cannot be given since males and subadults were not identified. To be able to determine the physiological state of a female, her precise identification was of crucial importance. Gestation was established in the data retrospectively after the birth of the young. Lactation was determined through the presence of a dependent offspring. However, growing up the offspring becomes increasingly independent from the mother; also, the offspring can disappear, die or be rejected by the mother (Clarke *et al.* 1998). Accordingly, we verified the lactation status of females with young offspring at least once a week.

Field work was carried out on Agaltepec Island with an area of 8.3 ha (18° 24' - 18° 25' N, 95° 05' - 95° 06' W) located in Lake Catemaco, Veracruz, México (Figure 1). The island includes flora of 4 vegetation types: medium subdeciduous forest, riparian vegetation, secondary vegetation and grassland, with a mean canopy height of 15–20 m.

In order to familiarize ourselves with the study animals the females were closely observed during data acquisition. The descriptions of all visible attributes of each female were recorded with a dictaphone and aggregated in a table. Females with very similar characteristics were juxtaposed in the table to segregate distinctions. Each female was followed under recording of her characteristics until an identification was possible, or until all characteristics were captured. This table gave us the advantage of identifying the focal animals *a posteriori* in case of uncertainty about the female's identity, which was unfortunately the norm. Additionally, photographs of the females were assigned to their characteristics for visualization.

Methodology of observation and description of the females. Division by sex and age. The most trivial distinction was the sub-division into sex groups. Mantled howler monkeys show sexual dimorphism and can be easily divided into females and males. Adult males are bigger, with clearly visible testicles, larger beards and larger upper carnassial teeth (Kelaita *et al.* 2011). The genitals are very well visible from the bottom view. However, young male subadults who did not reach their full growth can be easily mistaken for young females as the not fully developed testicles are not as evident. Therefore, special attention was given to young females for a proper association with the right sex.

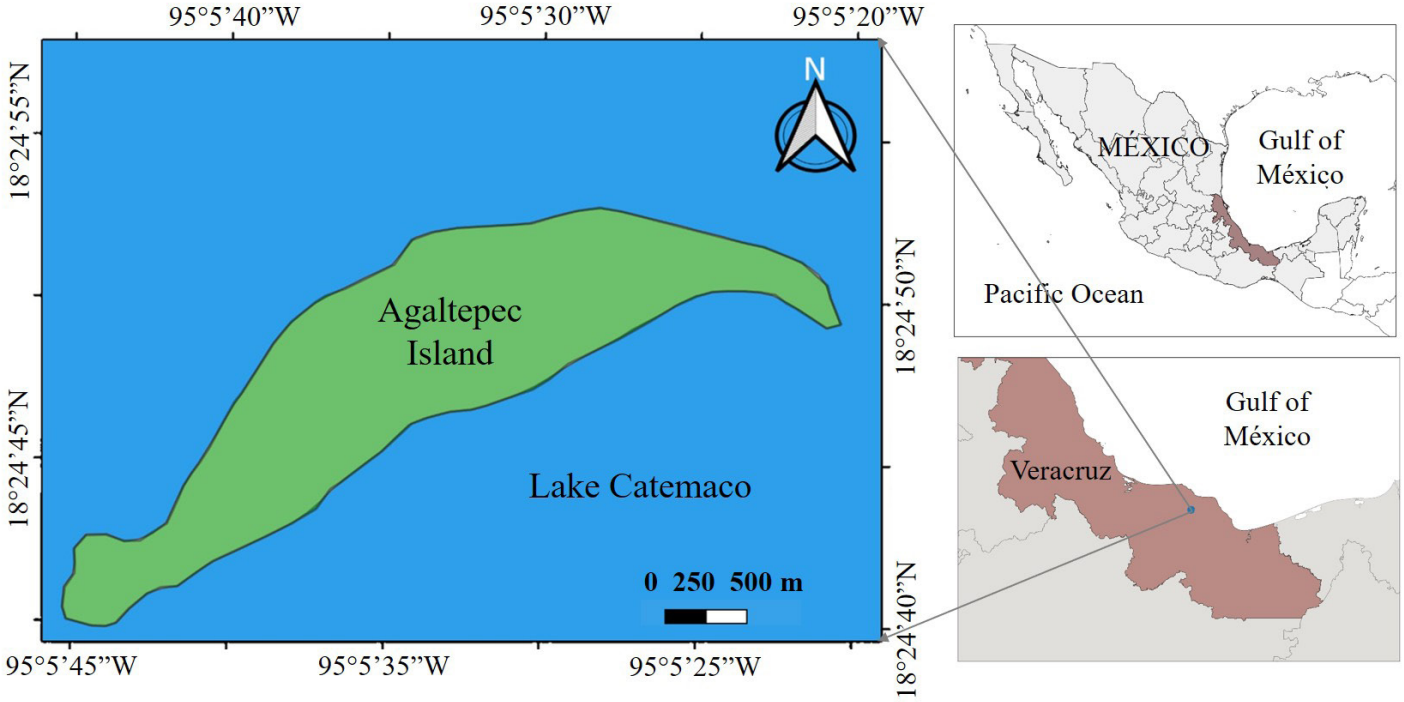

Figure 1. Localization of the Agaltepec island in the Lake Catemaco, Veracruz, México.

Most adult females were already mothers which resulted in their vulva being slightly bigger with enlarged labia (Figure 2a). Further, after nursing an infant, the breast nipples remained longer and the skin on breasts stretched and remained saggy (Figure 2b). These features were used for identification.

Deformities and injuries. Strong characteristics were missing or deformed body parts and facial features. Permanent damages like missing fingers and toes, ripped lips, ripped nasal wings and ears or missing eyes were the most obvious attributes on an individual.

Pelage. Some individuals can differ in the shade of their pelage color which will make them stand out from other monkeys. Yet, the pelage-hue can vary strongly from different angles of insolation. Subsequentially, the color of the pelage alone was not a reliable characteristic for identification and was not taken into account.

Marks. In mantled howler monkeys on the Agaltepec island, the coloration occurs on hands, feet and the tail and is blond to dark gray. We considered the colors, shapes and sizes of the patterns, as well as their consistence. The marks were small spots in some areas, bigger marks, colorations consistently covering a big expanse or inconsistent coverings with breakthroughs (Figure 2c). Descriptions comparing the markings with objects or occurrences, such as "the coloration on the tail resembles flames" were extremely helpful.

Markings on hands and feet. The colorations occurred on the soles and palms as well as fingers and toes, for the latter two also on the dorsal sides. However, coloration on the hands was rare, existing marks gave an individual a unique identifying feature. Feet are generally more visible than hands due to howlers' arboreal lifestyle and tend to show more patterns. Coloration on the dorsal side of the feet is unique and was given enormous attention and accuracy in description.

In some cases, the marks on fingers and toes were very subtle and resembled rings or painted nails. Extensive coloration on fingers and toes often occurred in a pattern that ranged over several limbs (Figure 2d).

Markings on tail. The tail of the howler monkey is generally visible to the observer. The most distinctive part on the tail is the bare patch of skin at the underside. This part often showed a coloration that varied from a line to complete filling with tidy, ripped, thick or subtle edges (Figure 2e). The pelage around the patch was occasionally colored as well consistently or inconsistently covering large or small areas of the tail. Frequently, the consistent marks had smaller marks and breakthroughs or patterns within (Figure 2f). Therefore, the tail was examined and described in very particular details.

Image documentation. The importance of photos and sketches must be stressed out. The abundance of photos of the same individual from different angles trains the eye to recognize this animal faster behind the veil of canopy. The complex patterns on tail and feet could be observed in detail from pictures for a better recognition in the field.

For a complete identification with all attributes, each female had to be closely observed and re-identified on different occasions for an average period of 1 month. Whenever possible, photos were taken with a high-resolution camera. With the ongoing process it was possible to iden-

Figure 2. Identification characteristics of female howler monkeys (*Alouatta palliata mexicana*) on the Agaltepec island. a) Bottom view of an adult female with an enlarged vulva who has already given birth. b) Enlarged breasts and nipples of a female that is currently nursing a dependent infant. c) Female 5 – Flame. Bottom view of the tail: coloration with breakthroughs with a "flame-like" pattern covering a large part of the tail. d) Female 10 – Triangula. Female with a distinct coloration on the left toes which enlarges from index to little toe resembling a "triangle". e) Naked patch of skin on the bottom side of the tail. The patch has a bright, longitudinal coloration and thick, uneven contours. f) Female 15 - Pointy. Female with a blond coloration around the tail which consists of a small, black mark.

tify and describe more and more females including the reserved and younger individuals. These targeted observations allowed us to describe 23 females in total.

Some females have been given very illustrative descriptions, as we perceived the resemblance to objects or occurrences. All marks, spots, dots, pattern and colorations referred to were of bright, blond color, unless mentioned otherwise.

Females 1–6 did not show signs of previous motherhood such as enlarged nipples or enlarged vulva, but have been observed copulating. Females 7–13 were with signs of previous motherhood, but have not been observed with an offspring. Females 13–23 have been observed with an offspring during the 24 months of the study. See Appendix 1 for descriptions and abbreviations.

The Agaltepec island is subject to investigations on mantled howler monkeys since their release in 1987 (Rodríguez-Luna *et al.* 1993). Generally, the investigations were not conducted on strictly identified animals (Asensio *et al.* 2007; Dias *et al.* 2008). However, since the animals are spatially restricted and do not migrate to other habitats, it makes the island suitable for comparison investigations and long-term projects with highly targeted questionings.

With our project we set a milestone for more targeted research on identified individuals for further generations of field biologists on the Agaltepec island. However, our projects focused solely on adult females, which consequently resulted in an identification list of only females. We hope the next generation of field biologists will describe the resident males in as much detail as we have done for the females.

For this matter they will have to overcome certain obstacles, such as the detection of the features despite the high distance and the occultation of the animals. Howler monkeys are at all times in the canopy of the trees even if fully habituated, and therefore often covered by vegetation. This highly arboreal lifestyle postulates the main problem of a successful re-identification, researcher in the past likewise encountered difficulties (Pope 2000). Due to the distance, solar rays on the pelage may give an illusion of a coloration and lead to confusion of the observer. The use

of high-definition binoculars (at least 8 x 42) which correct chromatic abbreviation is therefore essential.

Some of the described females have very similar marks which lead to mis-identification in the beginning (Prima/Rosa, Nini/Pointy, Rosa/Linda, Prima/Jamie). Also, due to allomaternal care which is widespread within the genus *Alouatta* (Calegaro-Marques and Bicca-Marques 1993) there was no strong reliance on the motherhood of a female. These facts highlight the strong importance of capturing and describing all the present features in details, and the importance of a detailed identification list.

This list can be highly supportive for an efficient re-identification of resident adult female mantled howler monkeys on the Agaltepec island. Herewith, we hope to help aspiring researchers by giving ideas how to start and maintain an effective identification process.

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Appendix 1

Detailed descriptions of 23 identified howler monkey females on the Agaltepec island. Abbreviations: Toes: T1 – great toe; T2-T5 respectively, with T5 being the outermost toe. LFD – left foot dorsal. LFV – left foot ventral. RFD – right foot dorsal. RFV – right foot ventral.

Female 1 – Luna

LFD: Coloration on all toes from the base to the middle joint. LFV: Chaotic coloration throughout the sole consisting black dots resembling paint brush strokes. RFD: Marks resembling rings on all toes except T3. RFV: Uneven coloration on the forefoot reaching from the base of the toes to the midfoot. The right site of the coloration contains a black mark. Tail: Extensive coloration on the patch with black, uneven edges resembling a gearwheel. Above the patch in the pelage is a rectangular pattern. This pattern resembles a flagpole with a flag to the left site. On the lower end of the tail in the pelage is a horizontal, thin mark circling the tip of the tail (like a bracelet) with a breaking on the dorsal part.

Female 2 – Jamie

LFD: The general color on the toes is grey; a horizontal mark runs over the middle joints, including T1. LFV: Coloration on the forefoot. RFD: T2 completely colored, T3 uneven coloring, T4 a stripe in the middle and on the nail. RFV: Uneven coloration from forefoot to midfoot. Tail: The patch has a thin, vertical mark in the middle and black, thick edges. In the pelage above the patch is a horizontal marking resembling a crotchet in width lager than the patch.

Female 3 – Ozomatli

LFD: No coloration. LFV: From forefoot to the end of midfoot are two marks separated by a black line. RFD: Big mark on the forefoot with a black point on the right site. RFV: No coloration. Tail: Above the patch begins a triangular mark in the pelage, which reaches to the middle of the patch, encasing it. The patch has a thin, vertically coloration through its middle and dark, uneven edges.

Female 4 – Velvet

No markings. Her pelage and skin are evenly brown on all extremities.

Female 5 – Flame

The coloration on the tail of this female is very distinct and is the reason for her naming. LFD: No coloration. LFV: Mark on the forefoot with a black point under T1. Around T1 runs a mark resembling a ring. From the lower site of the foot, oval-shaped markings reaching into the coloration on the forefoot. RFD: T3 mark on the middle joint. T4 mark from the base to the middle joint. RFV: Coloration over the whole foot with elongated black mark in the middle and a big black mark on the heel. Coloration on all toes, T1 with a horizontal, black breakthrough in the middle. Tail: On the dorsal part of the tail is a horizontal stripe resembling the letter "M". In the middle of the patch is a thin, vertical coloration with uneven contours. In the pelage above the patch is chaotic coloration going almost to the base of the tail and to a certain degree resembling flame flicker of a campfire (Figure 2c).

Female 6 – Prima

Extensive coloration on toes of both feet. LFD: T1 coloration on the base, T4 and T5 completely colored. LFV: The upper half of the foot colored with breakthroughs, the heel is black. RFD: Broad stripe over all toes, like a broad-brush stroke. The tips are black. RFV: T3 and T4 colored. In the middle of the sole uneven coloration with breakthroughs. Tail: The patch itself is completely black. The coloration of the pelage on the tail is grey.

Female 7 – Candle

Pattern on the patch of this female is the reason for her naming. No coloration on the dorsal sides of her feet. LFV: Small mark under T2. RFV: Uneven, chaotic coloration on the forefoot. On the outer edge of the foot is a thin, vertical mark with breakthroughs running to the heel ending in a swing over the heel. This pattern resembles a golf club above the black heel. Tail: The patch has a large coloration that narrows in width in the upper end resulting in a very slim trail. This trail is bent and resembles a burning wick.

Female 8 – Sugar

No coloration on the dorsal sides of her feet. LFV: An extensive coloration with a black spot in it. RFV: Two large marks on the forefoot: underneath T1 and under the remaining toes, separated by a thick, black line. Tail: The patch is largely colored, with thin, black edges.

Female 9 – Linda

No coloration on the dorsal sides of her feet. The ventral sides of both feet have similar colorations. LFV: Two horizontally elongated marks on the forefoot ending in the midfoot. RFV: Big coloration on the forefoot under T3-T5. Under T1 an oval

mark with a black point to the right side. Tail: The patch has a large coloration with uneven, black edges. The coloration is unevenly interrupted in the upper end of the patch. The pelage on the tail is gray, with strongly visible gray coloration from the ventral site.

Female 10 – Triangula

The coloration on the toes of the left foot is responsible for the naming of the female. No coloration on the right foot of this female, neither dorsal nor ventral. LFD: A coloration resembling a triangle going from T5, that is completely covered to the base of T2 with only a thin line over the base (Figure 2d). LFV: Large coloration with breakthroughs covering the whole sole including all toes. Tail: Large coloration covering the patch.

Female 11 – Anillo

No coloration on the feet of this female, neither dorsal nor ventral. Tail: The patch has a coloring with a ring-like black pattern on the upper end.

Female 12 – Patch

No coloration on the left foot of this female, neither dorsal nor ventral. RFD: No coloration. RFV: A small mark on the heel. Tail: Large coloration covering the whole patch.

Female 13 – Hilo

No coloration on the left foot of this female, neither dorsal nor ventral. RFD: No coloration. RFV: Small, uneven coloration on the forefoot. The tip of T3 is bright. Tail: A very thin, uneven, vertical coloration through the middle of the patch.

Female 14 – Nini

LFD: T5 and T4 colored, T3 colored from the base to the middle joint. LFV: Large, uneven coloration on the whole sole except the heel. Under T1 on the forefoot a mark with a black spot in it. All toes colored. RFD: Coloration on T4 and the tip of T5. RFV: No coloration. Tail: Colored patch with uneven, black contours. On the pelage around the tail is a chaotic, uneven, coloration inclosing the coloration of the patch.

Female 15 – Pointy

The pattern on the tail of this female is responsible for her naming. No coloration on the dorsal sides on both feet. LFV: T1-T5 colored. T1 a black line on the base. T3 and T4 with bright tips. The heel is colored and contains a diagonal, elongated black mark. RFV: Uneven coloration on the forefoot. Tail: In the patch large coloration with uneven, black edges. On the pelage around the tail is a mark going evenly around it like a bandage and enclosing the upper patch end. On the dorsal site of the bright pattern is a black dot (Figure 2f). Left hand ventral: Small markings on the finger pad.

Female 16 – Rosa

LFD: The toes unevenly colored with black tips. LFV: Uneven coloration on the whole sole. RFD: The toes unevenly colored with black tips. RFV: The coloration covers the whole sole but less extended than on the left foot. Tail: A very thin, vertical mark through the middle of the patch, making the patch appear almost black. The coloration of the pelage is gray.

Female 17 – Nova

LFD: T1 mark on the tip. T3 mark on the base. Coloring on T4 and T5. LFV: Uneven coloration on the whole sole except the heel, with a distinct spot in the middle of the sole and an elongated, diagonal mark over the heel. RFD: T1 colored. T2 mark on the tip. RFV: Forefoot colored. Above the heel a white mark. Tail: Vertical coloring through the middle with equally bright black edges. Above the upper end is an uneven mark resembling a star.

Female 18 – Honey

No coloration on the dorsal sides on both feet. LFV: Two elongated marks beginning at the forefoot and ending at the end of the mid foot, which gives an impression of a heart. Above the heel is a small, round mark. RFV: Two marks on the forefoot separated by a black line. A vertical elongated mark runs from T5 along the outer edge of the sole with a swing over the heel. Tail: Patch largely colored with black, uneven, thin edges.

Female 19 – April

No coloration on the dorsal sides on both feet. LFV: All toes colored. The sole is largely colored with random black spots in it. The heel is black. RFV: Two accurate marks on the forefoot separated by a thick, black, vertical line. Coloring on the tip of T1. Tail: The patch is mostly dark, with an uneven, thin, vertical mark in the middle. The mark includes three small, round shaped patterns in its upper half.

Female 20 – Bridgit

No coloration on the right foot of this female, neither dorsal nor ventral. LFD: No coloration. LFV: Two large marks: under T3-T5 and T1. One round spot above the heel. Tail: Thin, vertical mark in the middle of the patch, same width as each black edge. The black edges are uneven. Toward the upper tip of the patch the coloration narrows in width. The narrow part

consists of two horizontal, black lines (bridges).

Female 21 – Corta

No coloration on the dorsal sides on both feet. LFV: Narrow marking on the upper half of the forefoot. RFV: Extensive coloration on the upper half of the sole with many breakthroughs. All toes colored. Tail: The patch is largely colored. However, on the upper end of the patch the coloration seems "cut off", with a flat, abrupt ending on the top.

Female 22 – Noviembre

The most distinctive characteristic of this females is her left eye. The left eyelid is always partly closed. It is unknown if this feature is a result of an injury. No coloration on the dorsal sides on both feet. LFV: Two very flat, horizontal markings on the forefoot just under the base of the T3-T5 and T1. RFV: A spot on the outer site of the heel. Tail: Thin, vertical coloration in the middle of the patch. Toward the upper tip of the patch the coloration narrows in width.

Female 23 – Septiembre

The most distinctive characteristic of this females is the absence of T2-T4 on the left foot. No coloration on the dorsal sides on both feet. LFV: Few random markings on the forefoot. RFV: One mark on the forefoot. Tail: Patch largely colored with uneven, black edges.