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Research article

Assessment of the utility of a commercial calprotectin and lactoferrin rapid test in diagnosis of marmoset wasting syndrome

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Abstract

This study assessed the detection of faecal biomarkers in captive callitrichids affected by Marmoset Wasting Syndrome (MWS), using a commercial coloured chromatographic immunoassay intended for the detection of calprotectin and lactoferrin in humans affected by inflammatory intestinal diseases. The test was applied to faecal samples from 77 animals of 11 callitrichid species. Animals were divided into two groups consisting of 23 animals affected by MWS and 54 apparently healthy animals. All samples tested negative for lactoferrin while 64.9 % tested positive for calprotectin. The commercial test exhibited a high sensitivity (95.6%) but a low specificity (48.1%) for calprotectin, indicating poor utility in detecting new cases of MWS in a given population. A semi-quantitative assessment of the commercial test revealed a statistically significant difference between affected and non-affected animals for calprotectin (25.10/9.53, W=226, P=4.389e-05). Although the use of fecal biomarkers in the context of wasting syndrome did not seem to be completely conclusive, it would be interesting to investigate further as some trends were noticed in this study.

Introduction

Marmoset Wasting Syndrome (MWS) is an important disease in callitrichids under human care, both in zoos and laboratories (Ialeggio and Baker 1995). Its occurrence in laboratories has become rarer; however at least 10% of zoo housed animals may still be affected (Cabana et al. 2018). Prevalence of MWS seems not to be correlated to gender (Shimwell et al. 1979; Murgatroyd and Chalmer 1980; Zöller 2005) although some studies emphasise a potential association with dominant females (Quohs 2003; Winkelman 2010). However, even if the disease is not limited to one genus within the Callitrichidae family, prevalence seems to be species-specific according to Cabana and Maguire (2016) ranging from 0% for the golden headed lion tamarin (*Leonthopithecus chrysomelas*) up to 50% for the black-eared marmoset (*Callithrix penicillata*).

Clinical signs of MWS are diverse but the most common clinical picture is a combination of rapid weight loss, localised alopecia, chronic or intermittent diarrhoea and muscular atrophy (King 1976; Tribe 1979; Morin 1983; McNees et al. 1983). This disease is considered multifactorial and the causes are still hypothetical. The most frequent hypotheses proposed are nutritional deficiencies, behavioural stress and pathogens (Sainsbury et al. 1992; Ialeggio and Baker 1995; Quohs 2003). Nutritional deficiencies are believed to be less involved than previously thought. Inflammation in the gut prevents absorption of nutrients, giving rise to deficiency symptoms; the focus of MWS has shifted to identifying what causes this inflammation in the first place (Cabana et al. 2018).

There are several similarities between MWS and inflammatory bowel disease (IBD) in humans (Sainsbury et al. 1987; Bongard 2005). IBD is defined both in human and

veterinary medicine as a chronic and uncontrolled inflammation of the digestive tract (Hanauer 2006; Cerquetella et al. 2010) associated with a malabsorption syndrome that explains the majority of the clinical signs observed (Hanauer 2006; Cerquetella et al. 2010; Jarcho et al. 2013).

Efficient and easy diagnostic methods have been developed for the detection and the monitoring of IBD in humans. Calprotectin and lactoferrin are two inflammatory markers that are found to be highly concentrated in faeces of patients with IBD. They are stable in faeces for up to one week at ambient temperature (Røseth et al. 1999; Kopylov et al. 2014) and can tolerate several freeze-thaw cycles (Iskandar and Ciorba 2012). The detection and measurement of the markers are considered reliable and easy to process (Mendoza and Abreu 2009; Lewis 2011; Iskandar and Ciorba 2012). Therefore, it is reasonable to hypothesise that these biomarkers could be useful in the context of MWS diagnosis in callitrichids.

Rather than testing for the presence or prevalence of MWS, the aims of this study were, firstly, to establish the clinical utility of using a commercial test for the detection of faecal calprotectin and lactoferrin, as a diagnostic method for MWS in a population of callitrichids with animals suspected and non-suspected of MWS. Secondly, to assess its ability to discriminate between the healthy animals and animals suspected of MWS within the same population.

Materials and methods

Ethical approval

This study was approved by the Royal Dick School of Veterinary Studies Veterinary Ethical Review Committee (VERC), as well by the participating zoos prior to the beginning of the project.

Study design

The study involved 85 callitrichids from 11 species (Table 1). These animals were from three different zoological institutions: Wildlife Reserve Singapore zoos (Singapore) (n=70), Citadelle de Besancon (France) (n= 7) and Zoo Lyon (France) (n= 8). Across all species, 39 animals were male and 46 were female, and age ranged from 4 months up to 21 years.

All animals were housed, fed and managed as per recommendation of the EAZA husbandry guidelines for the Callitrichidae (Bairrão Ruivo 2010) edited by the European Association of Zoo and Aquaria to which the three zoos are members.

The animals were classified in two populations: suspected of MWS (S) and not suspected of MWS (N). An individual was considered as suspected (S) when it showed the classical clinical signs of MWS: weight loss associated with chronic idiopathic diarrhoea and a messy or greasy coat with the possibility of localised alopecia. Also suspected animals exhibited diarrhoea despite being negative for faecal parasite, *Campylobacter, Cryptosporidium, Giardia, Salmonella, Yersinia, Shigella* and their blood parameters were within the norms for the species.

Four females were excluded because of their pregnancy/ abortion state that may have influenced the expression of calprotectin and lactoferrin (Konikoff and Denson 2006; Grellet et al. 2014). For similar reasons, three individuals aged one year or less were also excluded (Dorosko et al. 2008; Grellet et al. 2014; Hestvik et al. 2011). This resulted in a study population of 77 individuals. According to our inclusion criteria, 23 were considered as suspected of MWS (S) and 54 were not suspected animals (N). Within the MWS suspected animals, two were from the *Callithrix* genus (n=2/12) and 21 from the *Saguinus* genus (n=21/47). None of the *Leonthopithecus* (n=0/14) and the *Callimico* (n=0/4) genus were suspected of MWS in this studied population.

Sample collection and testing

Seventy-seven fresh faecal samples were collected from the ground of the animal's enclosures or of their nest boxes or in the pet carrier when brought to the hospital for health check. The samples consisted of an entire faecal bolus for each animal. Samples were collected in a sterile container after isolation or observation of each individual in order to be sure of their origin. Samples were collected between October 2016 and July 2017.

A commercial diagnostic test was used to assess the presence of calprotectin and lactoferrin in primate faeces, consisting of a combo card using coloured chromatographic immunoassay for semi-quantitative detection of human calprotectin (hCp) and human lactoferrin (hLf) (CerTest Calprotectin + Lactoferrin® rapid test, CerTest, Biotec, Spain). A positive result is indicated by the appearance of a red band when the threshold of 500 ng/mL (50 µg hCp/g faeces) for calprotectin and 100 ng/mL (10 µg hLf /g faeces) for lactoferrin is reached. According to the manufacturer the intensity of the band is correlated with the concentration of the biomarker.

Each faecal sample was processed according to the instructions given by the manufacturer. Samples were processed within a maximum of one hour after collection. The Calprotectin+Lactoferrin combo card test was kept at room temperature and removed from its sealed bag just before using it. The faecal material was collected from the faecal bolus with the collection tube provided in the test kit. The sample was then dispersed in a diluent included in the collection tube and four drops of the mixture was dispensed in the dedicated area of the card test. The card was left on a flat surface for 10 minutes before reading it.

Since the rapid test CerTest Calprotectin + Lactoferrin® is a semi-quantitative test based on colour chromatography, the intensity of the colour response to the test was analysed. In order to assess objectively the differences in red colour intensity visible

Table 1: Summary of the sample population included in the study to assess calprotectin and lactoferrin in diagnosing marmoset wasting syndrome in zoo housed callitrichids (Wildlife Reserves Singapore, Zoo Lyon, France and Citadelle de Besancon, France).

Scientific name	Common name	Male	Female
Cebuella pygmea	Pygmy marmoset	2	0
Callithrix jacchus	Common marmoset	1	0
Callithrix penicillata	Black-tufted marmoset	3	4
Callithrix geoffroyi	White-headed marmoset	0	2
Callimico goeldi	Goeldi's marmoset	2	3
Saguinus imperator	Emperor tamarin	6	5
Saguinus midas	Red-handed tamarin	5	9
Saguinus bicolor	Pied tamarin	3	3
Saguinus oedipus	Cotton top tamarin	10	13
Leonthopithecus rosalia	Golden lion tamarin	1	1
Leonthopithecus chrysomelas	Golden-headed lion tamarin	6	6

to the naked eye, a method adapted from the "red, green, and blue" (RGB) method described by Gerald et al. (2001) was used. Each card test (positive and negative) placed on a flat surface was photographed from above at a 90-degree angle at a distance of 20 cm using a digital camera with a 18 mega pixels resolution under standard indoor fluorescent lighting conditions throughout the different locations. All the JPEG pictures were opened in Photoshop® (Adobe Photoshop CS6 version 13.0.0.0) and the image mode was set to RGB colour model. An area of 3000 pixels was selected at the place where the reactive strip appeared or is supposed to appear, using the "Marquee" function that allows the selection of a specific area in a picture. The hue was assessed with the "Histogram" function in order to obtain the value (integer numbers in the range 0 to 255, within a single 8-bit byte) for each RGB colour and also the mean value of the three colours. The difference between the red colour value (R) and the mean value were calculated in order to obtain a score for each test (Appendix 1).

Statistical analysis

In order to evaluate the usefulness of the CerTest Calprotectin + Lactoferrin® rapid test under our conditions, all the results obtained were summarised into a 2x2 table in order to determine the specificity (Sp), sensitivity (Se), positive predictive value (PPV) and negative predictive value (NPV).

The red dye scores were compared between N and S groups respectively for males and females. The series of data for each group were tested with a Shapiro test to test the normality of their distribution followed by a Fisher test (F-Test) for testing the equality of variances between two series of data.

As none of the series showed a normal distribution associated with an equality of their variances, the two sets of data were compared using a Wilcoxon Rank Sum test.

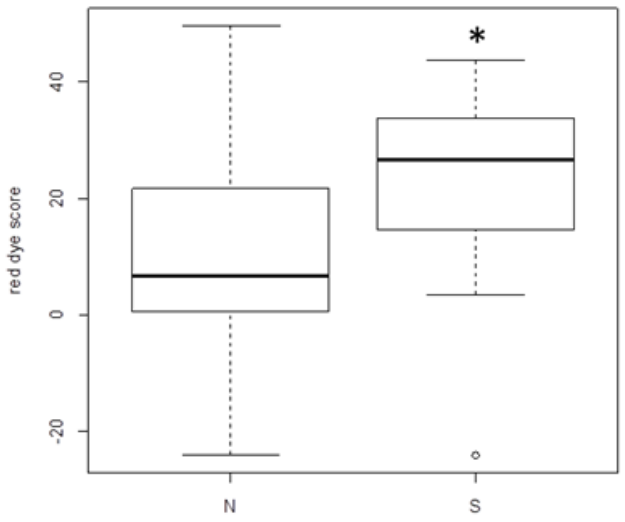

Figure 1: Red dye score of the CerTest Calprotectin + Lactoferrin® rapid test between non-suspected (N) and suspected (S) callitrichids. S: suspected, N: non-suspected. The semi-quantitative assessment of the commercial test revealed a significant difference between affected and non-affected animals for calprotectin (25.10/9.53, W=226, P<0.001).

Results

Clinical utility

Of the 77 samples tested, 50 (64.9%) animals were positive for presence of calprotectin in their faeces. All samples tested negative for lactoferrin. Within the two populations of animals, 95.6% (22/23) of suspected and 51.8% (28/54) of non-suspected animals tested positive (Table 2).

Semi quantitative assessment

Of the 77 digital images of the red colour band, three were not analysed due to poor quality and failure to assess them correctly on Photoshop®, resulting in 22 pictures for the S group and 52 for the N group (Appendix 1). No significant differences were found between males and females both for N (W=303, P=0.53) and S groups (W=69, P=0.60).

The mean score for red dye was 9.53 (±15.93) for N animals and 25.10 (±11.81) for S animals. The score for the S group was significantly higher than the N group (W=226, P<0.001) (Figure 1).

Discussion

The aim of this study was to assess a non-invasive commercial test used in human medicine for detecting faecal biomarker in patients with IBD, in callitrichids. The main hypothesis was that callitrichids considered suspect of suffering from MWS are affected by an intestinal inflammatory process (Jarcho et al. 2013), and thus inflammatory markers such as calprotectin and lactoferrin should be found in their faeces, as seen in humans affected by IBD (Hanauer 2006; Cerquetella et al. 2010).

The CerTest Calprotectin + Lactoferrin® rapid test was designed for human patients. It is a coloured chromatographic immunoassay for the semi-quantitative detection of human calprotectin and lactoferrin, based on the reaction between CLP and LFR in faeces with the mouse monoclonal antibodies anti-human calprotectin and anti-human lactoferrin. The first limitation of this study could be an absence of cross reaction between human and callitrichid CLP and LFR and the mouse monoclonal antibodies, which could be a reason for the absence of reaction for LFR test. It seems not to be the case for CLP as several animals had a positive reaction on the CerTest Calprotectin + Lactoferrin® rapid test. The cross reaction between human and callitrichid molecules may be not consistent. Schroeder et al. (1999), mentioned a relative cross reaction between human and callitrichid immunoglobulin A. However, it was impossible for Bongard (2005) to find animals affected by MWS, positive for anti-gliadin and anti-endomysium antibodies, two markers of coeliac disease in human.

Table 2: Summary of the clinical utility parameters of the CerTest Calprotectin + Lactoferrin® rapid test in callitrichids suspected (S) and not-suspected (N) of suffering from marmoset wasting syndrome.

Test Results	S	N	Total
+	22	28	50
-	1	26	27
Total	23	54	77
Se	0.96	PPV	0.44
Sp	0.48	NPV	0.96

In our study, SE was high indicating that a positive test often occurs in animals with MWS. In contrast, Sp was low, denoting a weak ability to identify animals correctly as negative when not suspected as having MWS. Predictive positive value was low, indicating a high number of false positive cases, and limiting the value of the CerTest Calprotectin + Lactoferrin® rapid test for case-detection or diagnosis. However, it could nonetheless act as a useful screening test because of a very high NPV, indicating a low number of false negative individuals. Calprotectin assays have different performances in detecting IBD in humans. Depending on the test (ELISA or immunochromatography), sensitivity and specificity ranges between 71–100% and 67–89% respectively (Labeare et al. 2014). The CLP is considered sensitive but not specific for the differentiation of organic and functional disease of the gastrointestinal tract (Caccaro et al. 2012).

In order to show a reaction, the CerTest Calprotectin + Lactoferrin® rapid test needed a specific threshold concentration of each marker. The cut-offs were set to 500 ng and 100 ng per ml respectively for CLP and LFR. Choosing the best cut-off has been the subject of many debates. Dhaliwal et al. (2014) concluded that 500 ng/ml is satisfactory in terms of Se and Sp for CLP, but the results improved significantly with a cut-off of 1000 ng/ml. In their review of CLP and LFR in patients with IBD, Caccaro et al. (2012) stressed the heterogeneity of the cut-off choice between studies ranging from 60 to 2000 ng/ml for CLP and from 70 to 200 ng/ml for LFR. The cut-offs inherent to the CerTest Calprotectin + Lactoferrin® rapid test could explain both the absence of reaction to LFR and the high proportion of non-suspect animals showing positive results to CLP.

Nakashima et al. (2013) demonstrated recently that common marmosets (*Callithrix jacchus*) express CLP during intestinal inflammation. Calprotectin has been found in marmoset faeces in concentrations ranging between 12.6–33.6 ng/ml in healthy animals and up to 380.7 ng/ml in animals with chronic diarrhoea. Surprisingly, lactoferrin is not extensively studied in marmosets. There are only few mentions of it in studies about eyelid apocrine gland secretion in primates (Stoeckelhuber et al. 2004) or as a genetic marker in pluripotent cell differentiation (Schrimpf et al. 2017).

One noteworthy limitation of the present study was to base the results and interpretations on a protocol using a single test per animal. Indeed, CLP excretion is known to have strong variability over time. Calprotectin concentrations vary significantly between patients but also on a day to day basis (Kristensen et al. 2016). Marmosets show different concentrations of CLP even when considered healthy with measurements ranging between one to three times higher (Nakashima et al. 2013). However, this observation does not seem to have an important clinical implication. The results are also strongly impacted by the high number of animals considered non-suspect which have a positive result for faecal CLP. The criteria used in this study to determine which animal is suspected of MWS could be too restrictive and could have led to poor distribution between S and N groups. The usual clinical signs were the major criteria for considering an animal suspect, but callitrichids with MWS can exhibit a range of other clinical manifestations such as nephropathies, metabolic bone diseases or changes in haematological and biochemical parameters. As well, CLP could be an early predictive biomarker of MWS in these positive animals, before the appearance of any clinical signs as it is the case for IBD relapses in human (Konikoff and Denson 2006).

Also, a significant number of the animals with a positive result to CLP while not being affected by MWS, were from one zoo. Although zoos are following international best practices guidelines, differences in husbandry still exist and could be a potential explanation for the important number of false positive animal. Indeed, stress and nutrition are two potential causative agents leading to a generalised inflammatory process. Callitrichids are social animals and live in familial groups governed by an important hierarchical system and strong dominance scheme (Bairrão Ruivo 2010). These false positive animals were almost all housed in non-familial groups including mixed species with single individuals of two to three different species. Enclosure design is also an important stressor for callitrichids (Bairrão Ruivo 2010; Cabana et al. 2018). A poor environment or one where they cannot escape from human view are considered deleterious. Cabana et al. (2018) emphasised that having a predator species near a callitrichid enclosure is a risk factor for developing MWS, while having visual barriers such as heavily planted hedges are protective factors. Finally, enclosures that are too small or too poorly furnished to allow sufficient activity of the animals could be a risk factor. Indeed, inactivity is considered as a significant risk factor for IBD in humans (Mendall et al. 2016). An interesting study would be to assess these apparently healthy animals for a few months or years later and see if they could be potential candidates for developing MWS.

The RGB method used in the semi-quantitative assessment for the CerTest Calprotectin + Lactoferrin® rapid test, as described by Gerald et al. (2001) was considered as an interesting process to objectify dye intensity and permit a quantitative measure for each test. Despite the precautions taken in this study to normalise the process, this method was subjected to several limitations. Although the RGB method is considered reliable (Gerald et al. 2001), quality of the camera, light quantity and quality (Endler 1990), shooting distance and number of pixels used for the calculation might all affect the values obtained. The measures acquired by this method are thus only indicator values and cannot be considered completely reliable. However, it was then possible to demonstrate a statistically significant difference between MWS suspected and non-suspected animals, as is the case between healthy and IBD patients in human medicine. However, the correlation between intensity of dye of the positive red band and actual concentrations of CLP and LFR was not possible to calculate as it was only mentioned by the manufacturer without any other explanation. It would have been interesting to know if the relation between coloration and concentration is following a linear or a logarithmic curve to calculate precisely the concentration of CLP in the samples.

Conclusions

1-The results from this study confirmed the presence of calprotectin in callitrichid faeces and demonstrated a significant difference between MWS suspected animals and healthy animals in terms of response to a semi-quantitative test for calprotectin based on colour chromatography. It was not possible to detect the presence of lactoferrin in callitrichid faeces with this diagnostic test.

2-Despite a weak clinical utility in the context of this study, mostly due to a high number of false positive animals, this commercial test might be interesting in the context of detection and diagnosis of MWS. Indeed, it was good in detecting affected animals and therefore could be used as a preliminary screening test in addition to the current diagnostic methods.

3-Further studies are needed as the cut-off concentrations of the test and husbandry management of callitrichids could be two important parameters influencing the results.

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Appendix 1: Summary of results and data for calprotectin test and picture analysis for each individual callitrichid. S: suspected, N: non-suspected, CLP: calprotectin, R: red, G: green, B: blue, M: male, F: female.

				Picture analysis with RGB Method				
S/N	Species	Gender	CLP	R	G	B	Mean	R minus Mean
N	*L. chrysomelas*	F	-	111,18	127,58	167,37	135,38	-24,2
N	*L. chrysomelas*	F	-	115,1	134,82	147,28	132,4	-17,3
N	*L. chrysomelas*	F	-	106,57	123,21	137,93	122,57	-16
N	*S. bicolor*	F	-	162,43	180,15	181,93	174,84	-12,41
N	*S. bicolor*	M	-	109,38	113,49	132,17	118,35	-8,97
N	*S. bicolor*	F	-	100,15	102,06	113,54	105,25	-5,1
N	*C. goeldi*	M	-	143,47	153,83	148,09	148,46	-4,99
N	*S. bicolor*	M	-	120,86	118,05	135,21	124,71	-3,85
N	*C. goeldi*	M	-	155,7	155,7	155,91	155,77	-0,07
S	*S. oedipus*	F	-					
N	*L. rosalia*	M	-					
N	*L. rosalia*	F	-					
N	*S. oedipus*	F	-	105,76	105,26	105,32	105,45	0,31
N	*C .penicillata*	M	-	151,59	150,74	151,4	151,24	0,35
N	*L. chrysomelas*	F	-	123,76	122,18	123,88	123,27	0,49
N	*S. oedipus*	M	-	147,26	146,2	146,71	146,72	0,54
N	*L. chrysomelas*	F	-	127,14	124,95	127,63	126,57	0,57
N	*S. oedipus*	F	-	130,09	127,88	128,69	128,89	1,2
N	*C. goeldi*	F	-	141,24	139,78	138,13	139,72	1,52
N	*S. oedipus*	M	-	144,54	141,75	141,36	142,55	1,99
N	*L. chrysomelas*	M	-	121,31	117,12	117,2	118,54	2,77
N	*L. chrysomelas*	M	-	118,21	113,33	113,82	115,12	3,09
N	*S. midas*	F	-	136,91	132,02	131,37	133,43	3,48
S	*S. oedipus*	F	+	143,83	136,77	137,76	139,45	4,38
N	*L. chrysomelas*	F	+	142,49	135,59	136,11	138,06	4,43
N	*S. oedipus*	M	+	123,06	115,88	116,47	118,47	4,59
N	*C. penicillata*	F	-	121,06	112,67	111,94	115,22	5,84
S	*S. oedipus*	M	+	152,9	142,19	145,52	146,87	6,03
N	*S .imperator*	M	+	179,94	171,08	168,67	173,23	6,71
N	*S .imperator*	F	+	157,42	148,87	145,64	150,65	6,77
N	*S. oedipus*	M	+	195,87	179,91	181,52	185,76	10,11
N	*C. penicillata*	M	+	156,85	139,7	142,23	146,26	10,59
N	*S. oedipus*	M	+	180,71	163,5	162,05	168,75	11,96
N	*C. penicillata*	F	+	146,97	127,35	129,69	134,67	12,3
N	*C. penicillata*	F	+	163,5	144,93	143,64	150,69	12,81
N	*S. oedipus*	M	+	171,54	152,42	148,92	157,63	13,91
S	*S. bicolor*	F	+	177,5	153,56	158,27	163,11	14,39
S	*S .imperator*	M	+	209,73	187,14	188,97	195,28	14,45

Appendix 1 (continued): Summary of results and data for calprotectin test and picture analysis for each individual callitrichid. S: suspected, N: non-suspected, CLP: calprotectin, R: red, G: green, B: blue, M: male, F: female.

S/N	Species	Gender	CLP	R	G	B	Mean	R minus Mean
N	*S. oedipus*	F	+	166,99	143,74	142,56	151,09	15,9
S	*S. midas*	M	+	170,74	136,29	148,43	151,82	18,92
N	*S. oedipus*	M	+	217,05	191,15	185,77	197,99	19,06
N	*L. chrysomelas*	M	+	173,67	145,21	141,42	153,43	20,24
N	*L. chrysomelas*	M	+	212,15	177,62	183,45	191,07	21,08
N	*C. goeldi*	F	+	181,75	148,24	148,84	159,61	22,14
N	*C. penicillata*	M	+	177,31	142,98	141,54	153,94	23,37
N	*C. penicillata*	F	+	197,37	160,56	163,12	173,68	23,69
N	*S. imperator*	F	+	214,24	176,48	180,84	190,52	23,72
S	*S. midas*	M	+	211,95	175,18	176,44	187,86	24,09
S	*S. oedipus*	M	+	160,5	122,37	126,32	136,4	24,1
N	*C. pygmea*	M	+	184,79	144,97	151,39	160,38	24,41
S	*S. imperator*	F	+	215,84	175,82	181,19	190,95	24,89
S	*S. midas*	F	+	188,89	142,08	151,17	160,71	28,18
N	*L. chrysomelas*	M	+	198,56	156,93	155,22	170,24	28,32
S	*S. midas*	F	+	184,48	139,38	143,91	155,92	28,56
N	*C. pygmea*	M	+	215,97	170,38	175,1	187,15	28,82
S	*S. imperator*	M	+	180,67	135,82	138,43	151,64	29,03
S	*S. midas*	F	+	157,35	111,88	113,83	127,69	29,66
N	*S. oedipus*	F	+	223,06	173,12	180,5	192,23	30,83
S	*S .midas*	F	+	181,78	132,96	137,61	150,78	31
S	*S. bicolor*	M	+	178,21	134,8	125,42	146,14	32,07
S	*C. jacchus*	M	+	154,48	99,45	103,05	119	35,48
S	*S. imperator*	M	+	219,13	160,74	170,07	183,31	35,82
S	*S. midas*	F	+	178,1	121,68	126,39	142,06	36,04
N	*S. oedipus*	M	+	225,25	166,67	169,57	187,16	38,09
S	*S. midas*	M	+	216,33	153,43	164,05	177,94	38,39
S	*S. imperator*	F	+	190,5	133,99	131,21	151,9	38,6
S	*S. oedipus*	F	+	166,96	98	104,92	123,3	43,66
N	*S. oedipus*	F	+	192,1	123,09	126,25	147,14	44,96
N	*S. oedipus*	F	+	216,18	136,26	150,66	167,7	48,48
N	*S. oedipus*	F	+	224,86	145,52	155,62	175,33	49,53
N	*S. imperator*	M	+	158,68	144,19	151,22	151,36	7,32
S	*S. imperator*	M	+	177,66	172,69	174,81	175,05	2,61
N	*S. oedipus*	F	+	171,52	173,11	173,98	172,87	-1,35
N	*S. oedipus*	F	-	149,83	147,09	147,08	148	1,83
N	*L. chrysomelas*	M	-	179,36	177,04	182,4	179,6	-0,24
N	*C. geoffroyi*	F	-	155,79	152,97	153,25	154	1,79
S	*C. geoffroyi*	F	+	189,8	168,87	175,11	177,93	11,87