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1	<b>RUNNING HEADING: SHORT COMMUNICATIONS</b>
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3	FULL TITLE:
4	NON-INVASIVE BODY TEMPERATURE MEASUREMENT OF WILD
5	CHIMPANZEES USING FAECAL TEMPERATURE DECLINE
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## 26 ABSTRACT

27 New methods are required to increase our understanding of pathological processes in wild 28 mammals. We developed a non-invasive field method to estimate the body temperature of 29 wild living chimpanzees habituated to humans, based on statistically fitting temperature 30 decline of faeces after defecation. The method was established using control measures of 31 human rectal temperature and subsequent changes in faeces temperature over time. The 32 method was then applied to temperature data collected from wild chimpanzee faeces. In 33 humans we found good correspondence between the temperature estimated by the method 34 and the actual rectal temperature that was measured (maximum deviation 0.22°C). The 35 method was successfully applied and the average estimated temperature of the chimpanzees 36 was 37.2°C. This simple-to-use field method reliably estimates the body temperature of 37 wild chimpanzees and probably also other large mammals.

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Key-words: health monitoring; wild great apes; wild chimpanzees; body
temperature estimation; non-invasive method

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42 Disease plays an important role in the demography of great apes, and health 43 monitoring is a vital part of the conservation of these endangered species (Leendertz et al., 44 2006). In most cases, diagnosis of disease is restricted to observations of clinical signs and 45 laboratory analysis of samples collected non-invasively. The development of non-invasive 46 methods is therefore essential to improve health monitoring of wild great apes. Body 47 temperature can be measured non-invasively with infrared equipment, but such equipment 48 is expensive. Here we present a simple, inexpensive and reliable field method to estimate 49 the body temperature of wild chimpanzees non-invasively.

The study was conducted in the tropical rain forest of Taï National Park (5°15'-6°07'N, 7°25'-7°54'W) in Côte d'Ivoire, where wild chimpanzees (*Pan troglodytes verus*) have been habituated to human presence. First, the method was established by 29 control measures performed on humans (one male and one female). Thereafter the method was applied to 31 faecal samples collected from visually healthy chimpanzees (17 samples from nine females; 14 samples from seven males).

56 For the human control measures, the rectal temperature was measured prior to 57 defecation using a commercially available digital thermometer. Thereafter the data were 58 obtained from both humans and chimpanzees in the same way and as follows. The exact 59 time of defecation was recorded and approximately 100 grams of the faeces was collected 60 from the ground as soon as practically possible and held in a gloved hand. Only well 61 formed faeces deposited on the ground were included in the study. The sensor of the 62 thermometer (a digital rectal thermometer or a digital thermometer [Data-Logger 63 thermometer 306]) was placed in the middle of the faeces and held as still as possible 64 during the measurements. The temperature was recorded approximately every 20 seconds 65 or two seconds, with shorter intervals possible using the data-logging thermometer, over a 66 period of at least six minutes. After the measurements were taken the glove was reversed, 67 closed, and weighed with a digital scale. The net weight of the faeces and the 68 environmental temperature were recorded.

Measured temperatures occasionally comprised outliers or unrealistic values, e.g. temperatures rapidly decreasing and later increasing again. Such curves might arise when the sensor opens a hole in the faecal sample, or when the sensor moves outside the sample. To exclude such outliers and unrealistic values from analyses, we used the following operational rule: A measure was excluded if a larger value occurred later in the sequence of measurements (Figure 1). We used a sigmoidal equation to express temperature decrease as a function of time
since defecation. The model we fitted was:

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$$T_{(t)} = d + a^*(1/(1+e^{(t-b)/c}))$$

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80 where T indicates temperature, t the time since defecation, and a, b, c, and d are coefficients 81 needed to describe the sigmoidal curve. More information on the equation, and a program 82 to implement this method is freely available via the internet 83 (https://diseasegroup.eva.mpg.de/mediawiki-1.6.5/index.php/Defecation) or via email 84 contact with the authors. To evaluate the procedure we compared observed values of rectal 85 temperature with those predicted by the method and related the differences to several 86 covariates. We used standard non-parametric procedures chosen according to the rationales 87 described in Siegel & Castellan (1988) for statistical testing. Tests and curve fits were 88 calculated using SPSS 13.0.1 or a computer program written by R.M. for Spearman's rank 89 correlation. When small samples required their use, we applied exact tests (Mundry & 90 Fischer, 1998; Siegel & Castellan, 1988). Approximate P-values were based on 10,000 91 permutations for calculating the significance of Spearman's rank correlation coefficients.

92 Using the sigmoidal model on the human control measures we found a clear 93 relationship between the temperature estimated and the actual rectal temperature that was 94 measured (Spearman's rank correlation: r<sub>S</sub>=0.62, N=29, P<0.01; Figure 2). The average 95 (arithmetic mean) absolute deviation between estimates and actual rectal temperatures was 96 0.22°C (N=29). Fifteen of the estimates differed less than 0.12°C from the actual value, 97 whereas only three differed by more than 0.5°C from the rectal temperature. Estimated 98 temperatures did not obviously over- or underestimate rectal temperatures (Wilcoxon test: 99  $T^+= 262$ , N=29, P=0.35). Temperature estimates were not influenced significantly by

100 sample weight ( $r_s=0.08$ , N=26, P=0.68) or environmental temperature ( $r_s=0.08$ , N=30, P= 101 0.70). Absolute deviations of the estimated temperature from the rectal temperature were 102 neither related to sample weight ( $r_s$ =-0.13, N=25, P=0.53) nor to environmental 103 temperature ( $r_s=0.27$ , N=29, P= 0.16). However, accuracy of temperature estimation in the 104 human samples decreased with increasing interval between defecation and beginning of 105 measurement ( $r_s=0.46$ , N=29, P=0.01; Figure 3). The measurements started on average 106 1.42 minutes (=mean; range 0.17 - 3.17) after defecation. Note that after the thermometer 107 was first placed into the faecal sample, the temperature usually increased for several 108 measurements. We took the point at which the temperature reached its maximum as the 109 beginning of measurement. Accuracy decreased with decreasing number of measures obtained (r<sub>S</sub>=-0.37, N=29, P=0.05), whereas total duration of measurement interval did not 110 111 obviously influence accuracy (r<sub>s</sub>=0.06, N=29, P=0.75).

The estimated temperature of the chimpanzees ranged from 34.6 to  $39.5^{\circ}$ C (excluding one value of 44.3°C, which was clearly outside physiological range). The average temperature of 16 chimpanzees was  $37.2^{\circ}$ C (figure 4). We found no significant difference in estimated body temperature between males and females (U=22, N<sub>females</sub>=9, N<sub>males</sub>=7, P=0.35). For the females, the temperature generally increased during the day, whereas this difference was not obvious in the males (Figure 5).

The weight of faecal samples did not differ significantly between chimpanzees (median 97 grams; range: 40 - 172) and humans (median 99 grams; range: 23 - 156) (Mann-Whitney U-test: U=352.5, N<sub>chimpanzee samples</sub>=29, N<sub>human samples</sub>=26, P=0.68). The temperature measurements of chimpanzee samples started on average 0.58 minutes after defecation (= median; range: 0.18 - 6.00).

123 In summary, our non-invasive method allows reliable estimation of the body 124 temperature of wild living chimpanzees. The method is easy to perform and requires no expensive equipment, as only a commercially available digital thermometer is needed, in addition to subsequent access to a computer. In the human control measurements half of the body temperatures were estimated with an error of less than 0.12°C. To ensure the most accurate results from this method, it is important to start the measurements as soon as possible after defecation and to continue the measurement until the temperature curve "flattens out." The requirement for fresh faecal samples means, however, that the method is most applicable to human-habituated animals, such as the chimpanzees studied here.

132 The weight of the faeces did not influence the accuracy of the temperature 133 estimation. Only adult chimpanzees were included in this study, however, as their faeces 134 were most comparable in size to human faeces. The method has not yet been evaluated for 135 use in younger chimpanzees with relatively smaller faeces, or in other species. 136 Environmental temperature also did not obviously influence the accuracy of body 137 temperature estimates. Conditions could be somewhat different in other field sites with 138 different climatic conditions and habitats. If so, it would be useful to verify the correct 139 measurement technique by performing human control measurement in local conditions.

140 Normal human rectal temperature ranges from 34.4 to 37.8°C (Sund-Levander et al., 141 2002), which shows that even for humans it is impossible to assign a single value as 142 "normal" body temperature. We should therefore expect a similar variation in normal body 143 temperature in wild great apes. Indeed, most of the body temperature estimates of the 144 chimpanzees in this study were within the range of normal human temperature, with an 145 average of 37.2°C. The very low end of the temperature estimate range could be explained 146 by poor measurement techniques, although they are within normal range in humans. Any 147 results could be retested if doubts emerge concerning the measurements. The estimated 148 temperature should be evaluated together with behavioural data, since stress or activities 149 such as hunting or fighting potentially produce a temporary increase in body temperature. It might be useful to know the usual range for an individual when he or she is healthy, so that a comparison can be made when illness occurs. We did not find any difference between the temperature of males and females in this study, but the numbers of samples might have been too low to detect a difference. Our study also showed that, at least for females, body temperature tends to be lower in the morning than later during the day. More data are required to confirm this pattern in wild primates.

156 When assessing the health status of individuals, veterinarians and field workers in 157 many areas of great ape research could use temperature data to complement behavioural 158 and reproductive data. Body temperature measurements can also be linked to the study of 159 self-medication (Huffman, 2003) – e.g. to help identify the fever-reducing effects of certain 160 plants - and more generally to increase our knowledge about sickness behaviour in 161 primates, as few detailed reports are available (Nunn & Altizer, 2006). Lastly, data on 162 body temperature can help to investigate disease outbreaks and, more generally, will 163 increase our understanding of the effects of disease and fever on wild chimpanzees and 164 other animals.

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267 Females: r<sub>s</sub>=0.53, N=18, P=0.022; Males: r<sub>s</sub>=0.14, N=13, P=0.64.