Abstract

Many studies have demonstrated the importance of symbiotic microbial communities for the host with beneficial effects for nutrition, development, and the immune system. The majority of these studies have focused on bacteria residing in the gastrointestinal tract, while the fungal community has often been neglected. Gut anaerobic fungi of the class Neocallimastigomycetes are a vital part of the intestinal microbiome in many herbivorous animals and their exceptional abilities to degrade indigestible plant material means that they contribute significantly to fermentative processes in the enteric tract. Gorillas rely on a highly fibrous diet and depend on fermentative microorganisms to meet their daily energetic demands. To assess whether Neocallimastigomycetes occur in gorillas we analyzed 12 fecal samples from wild Western lowland gorillas (*Gorilla gorilla gorilla*) from Dzanga-Sangha Protected Areas, Central African Republic, and subjected potential anaerobic fungi sequences to phylogenetic analysis. The clone library contained ITS1 fragments that we related to 45 different fungi clones. Of these, 12 gastrointestinal fungi in gorillas are related to anaerobic fungi and our phylogenetic analyses support their assignment to the class Neocallimastigomycetes. As anaerobic fungi play a pivotal role in plant fiber degradation in the herbivore gut, gorillas might benefit from harboring these particular fungi with regard to their nutritional status. Future studies should investigate whether Neocallimastigomycetes are also found in other non-human primates with high fiber intake, which would also benefit from having such highly efficient fermentative microbes.

Keywords: gut microbiome, Neocallimastigales, gorillas, diet

Introduction

Symbiotic microbial communities residing in the intestinal tract, referred to as the gut microbiome, are assemblages of bacteria, fungi, protozoa, and archaea that provide crucial functions for host nutrition (e.g. Sekirov et al., 2010; Robert & Bernalier-Donandille, 2003), development (e.g. McFall-Ngai, 2002), and immune systems (e.g. Hooper et al., 2012; Round & Mazmanian, 2009). Since many

microbes collected from environmental samples are uncultivable (Torsvik & Ovreas, 2002), advances in culture-independent methods, particularly metagenomic approaches based on high-throughput sequencing, allow the detection of a far more detailed microbial diversity than traditional culture based approaches (e.g. Caporaso et al., 2012). These methods have led to an increased understanding of the factors shaping the composition of microbial communities. There is common agreement that the two main factors influencing the microbial community structure are host phylogeny and diet (e.g. Sanders et al., 2014; Muegge et al., 2011). For example, a study investigating the gut microbiome of 60 different mammal species shows that conspecifics harbor bacterial communities more similar to each other than to those of a different host species and that these communities cluster according to host taxonomy. Principal coordinates analyses also provide evidence for the significant impact of diet on gut microbiome structure, because bacterial communities cluster in accordance with diet and gut type (Ley et al., 2008).

Neocallimastigomycetes are obligate anaerobic fungi that were first isolated in ruminants (Orpin, 1975). Their occurrence has also been confirmed in various non-ruminant herbivores like African elephants (*Loxodonta africana*), horses (*Equus ferus caballus*), black rhinoceroses (*Diceros bicornis*), red kangaroos (*Macropus rufus*) and in the herbivorous green iguana (*Iguana iguana*) (Nicholson et al., 2010; Liggenstoffer et al., 2010; Mackie et al., 2004). Intestinal anaerobic fungi are remarkable in their capacities to degrade plant material that is indigestible by the host. They harbor highly efficient hydrolases (cellulases, xylanases, mannoses, esterases, glucosidases, and glucanases) aggregated in extracellular enzyme-complexes, termed cellulosomes. These fungal enzymes are assumed to exceed the fermentative capacities of bacterial enzymes (Lee et al., 2000). Additionally, anaerobic fungi are among the first to colonize plant fragments (Edwards et al., 2008) and are able to mechanically penetrate plant cell walls (Doi & Kosugi, 2004; Fontes & Gilbert, 2010). Due to this initial colonization of plant particles and the mechanical breakdown of large plant particles as well as plant cell walls

anaerobic fungi facilitate the accessibility to fermentable substrates for residential bacteria that take part in the hydrolisation of plant fiber in the gastrointestinal tract (Bauchop, 1981).

Currently, Neocallimastigomycetes include one order, Neocallimastigales, with one family (Neocallimastigaceae) that encompasses six long known genera (*Neocallimastix, Caecomyces, Orpinomyces, Piromyces, Anaeromyces,* and *Cyllamyces*) and three newly described genera (*Buwchfawromyces*: Callaghan et al., 2015; *Oontomyces*: Dagar et al., 2015 and *Pecoramyces*: Hanafy et al., 2017). However, studies of various herbivorous animals propose a revised taxonomy with several new groups (Tuckwell et al., 2005; Fliegerová et al., 2010; Liggenstoffer et al., 2010; Nicholson et al., 2010; Herrera et al., 2011; Kittelmann et al., 2012). Studies suggest that the abundance and composition of different anaerobic fungi genera are dependent on host taxonomy, type of gut fermentation, and fiber content in the diet (Liggenstoffer et al., 2010; Kumar et al., 2013; Denman et al., 2008).

Despite the growing number of studies investigating the gut microbiome in primates, the fungal community has received disproportionately little attention. Many early studies focused on specific mycotic infections (reviewed in Migaki et al., 1982), and a more recent study targeted a broader diversity of enteric fungi in Western lowland gorillas (*Gorilla gorilla gorilla*). This molecular survey of pathogenic eukaryotes detected 52 fungal species, all belonging to the taxa Ascomycota and Basidiomycota (Hamad et al., 2014). However, no study has yet investigated Neocallimastigomycetes in primates, even though there is good reason to hypothesize that some primates harbour these fungi. Most primates rely on a mainly plant based diet (Chapman & Chapman, 1990), yet, like all mammals, they lack the enzymes to degrade plant structural polysaccharides themselves and thus rely on endosymbiotic microorganisms for an adequate nutritional intake (Mackie, 2002).

Studies of gorilla feeding ecology reveal that they consume high fiber staple and filler fallback foods such as terrestrial herbaceous vegetation, figs, bark, and pith year-round (Western lowland gorillas: Remis, 2003; Doran-Sheehy at al., 2009). Although chimpanzees (*Pan troglodytes*) also consume high fiber plant material such as pith in times of fruit scarcity (Wrangham et al, 1991), there is strong support for the hypothesis that chimpanzees can maintain a higher quality diet with overall less fiber intake when compared to Western lowland gorillas (Tutin et al., 1991; Wrangham et al., 1998). In line with these observations, gorillas show morphological and physiological adaptations that suggest heavy reliance on high fiber foods. For example, their molar morphology indicates a high capacity for processing tough food (Ungar et al., 2007). Further, gorillas have an enlarged colon surface area and a longer mean gut retention time when compared to less folivorous chimpanzees (Chivers & Hladik, 1980; Milton & Demment, 1988; captive Western lowland gorillas: Remis & Dierenfield, 2004) even when accounting for body mass (Harrison & Marshall, 2011). Moreover, daily energy consumed that potentially originates from microbial fermentation in the hindgut is an estimated 57.3 % for western lowland gorillas and 24.7 % for chimpanzees (Popovich et al., 1997; Conklin-Brittain et al., 2006). Gorillas further fulfill two major prerequisites for the potential of harboring anaerobic fungi: a dedicated enlarged digestive chamber for microbial fermentation (hindgut) and a relatively long retention time for plant material.

We explore fungal communities in feces of wild Western lowland gorillas using culture-independent molecular methods. Specifically, we aim to amplify ITS1 rDNA fragments of Neocallimastigales from DNA isolated from fecal samples. Given their year-round exploitation of high-fibrous foods, we hypothesize that gorillas benefit from harboring highly efficient fermentative microorganisms such as anaerobic fungi in their intestinal tract. Based on their digestive morphology, we predict that it is very likely that Neocallimastigales are part of the gorilla gut microbiome.

Methods

Study site, subjects and sample collection

We collected fecal samples from two habituated groups of wild Western lowland gorillas at two field sites: Bai Hokou and Mongambe in Dzanga-Ndoki National Park, Dzanga-Sangha Protected Areas, Central African Republic, from September 2014 to January 2015. Both field sites comprise semi-deciduous forests and are characterized by seasonal variations in rainfall with a dry season lasting from December to February (for detailed description see Masi, 2007). We collected samples from known individuals as soon as possible after defecation, i.e. as soon as it was safe to collect the sample without disturbing the animal, which was usually within minutes.

We fixed fecal material in 96% ethanol in 8 ml tubes (approximate ratio 2/3 ethanol to 1/3 sample material) and stored the samples at ambient temperature at the field sites until we transported them to the University for Veterinary Medicine and Pharmaceutical Sciences, Brno, Czech Republic, where we kept them in ethanol at -20°C until analysis. We preserved fecal material in ethanol due to the lack of other storage possibilities at the field sites. DNA has been successfully isolated and amplified from such fixed samples (Frantzen et al., 1998; Hale et al., 2015) and preserving samples in highly concentrated ethanol at ambient temperatures appears to have little influence on the microbial community (Song et al., 2016).

Our study is a preliminary investigation for which we processed 12 gorilla samples, representing 11 individuals. We picked gorilla samples randomly from the samples we collected during the study.

Sample Processing

DNA Isolation. After evaporating ethanol at 40°C (heat block) overnight, we isolated DNA from the fecal material with the FastDNA^{TM} Spin Kit for Soil (MP Biomedicals, USA) according to the manufacturer's protocol with the following changes: to break fungi chitin walls, we homogenized the sample by bead-beating it three times for 30 sec at 6 m/s with 30 sec on ice between homogenization

steps (Cheng et al., 2009). We eluted DNA with 70 µl instead of 100 µl of the elution solution provided with the kit and stored eluates at -20°C.

PCR Amplification of Fungal Barcodes. We used the fungal universal forward primer ITS1F (CTTGGTCATTTAGAGGAAGTAA) in combination with a primer specific for anaerobic fungi NeoQ PCR R (GTGCAATATGCGTTCGAAGATT) to amplify ITS1 fragments (Fliegerová et al., 2010). We prepared PCR reactions with a final volume of 25 µl using the QIAGEN Multiplex PCR Kit (Qiagen, Germany) containing 12.5 µl Master Mix, 8.0 µl dH_2O, 2.5 µl dye 0.01 µM of each primer and 1 µl DNA. We set cycling conditions of the touchdown PCR protocol as 95°C for 5 min; 20 cycles consisting of 95°C for 30 sec, 60.5°C for 30 sec with -0.2°C per cycle, 72°C for 30 sec; followed by another 20 cycles consisting of 95°C for 30 sec, 57°C for 30 sec, 72°C for 30 sec and a final extension of 5 min at 68°C. We visualized PCR products on 1% agarose gels and subjected fragments of expected size to cloning procedure after purification with ExoSap (Affymetrix Inc., USA).

Cloning Library Construction. We constructed a clone library with the TOPO TA Cloning Kit for Sequencing (Life Technologies, USA) following the manufacturer's protocol for vector preparation and the transformation of competent *E. coli* cells. We picked 289 clone colonies and transferred them into 20 µl PCR H_2O to screen them for the presence of the insert by PCR. We prepared PCR reaction mixtures of 25 µl containing 12.5 µl Master Mix (PCRBIO Taq Mix Red, PCR Biosystems, UK), 9.5 µl dH_2O, 1 µl of clone colony solution and 0.01 µM of ITS1F and NeoQ PCR R primers. We set cycling conditions for ITS1 insert amplification as 95°C for 5 min, followed by 30 cycles of 95°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec and a final elongation for 5 min at 72°C. We checked PCR products using gel electrophoresis, purified products of the right length with ExoSap and subjected them to Sanger sequencing (Macrogen Europe, The Netherlands).

Sequence Analysis

We first edited sequences with BioEdit software (version 7.2.3) and subsequently used GenBank's Basic Local Alignment Search Tool (BLAST; default setting highly similar sequences (megablast)) to identify their nearest relatives. We only subjected sequences that we could relate to anaerobic fungi to further analysis. Given that sequence similarity among different anaerobic fungi strains can be very high (Goudarzi et al., 2015) we first aligned a selection of 12 clone sequences, as representatives for all related anaerobic fungi strains, to assess their resemblance (ClustalX, Bioedit; Hall, 1999; Table 1, Appendix S1). We subsequently chose a subset of the nine most divergent sequences for phylogenetic analysis to determine the taxonomic relationships of potential ape anaerobic fungi strains with known Neocallimastigales. By applying the MAFFT algorithm with default settings (online version 7, ©Katoh, 2013) we computed alignments that included ITS1 fragments generated in this study and reliable ITS1 sequences representing the improved taxonomic framework for Neocallimastigales fungi (Kittelmann et al., 2012; Dagar et al., 2015; Appendix S2). In addition to these reference sequences classified as Neocallimastigales we included the uncultured *fungus clone AFI-1* sequence isolated from Bactrian camel (*Camelus bactrianus*) rumen (Acc. No: JX944983). High degrees of sequence dissimilarities and length polymorphisms between Neocallimastigales genera resulted in multiple large gaps in the original 452 bp alignment. Given that the applied Maximum Likelihood algorithm treats gaps like missing data we aimed to reduce ambiguity by manually deleting those gaps to different degrees, resulting in two further alignments, one of 241 bp and another of only 197 bp.

We constructed phylogenetic trees in PhyML (Guindon et al., 2010) based on the original MAFFT alignment and two further alignments. Based on the results of Modeltest 3.7 (Posada & Crandall, 1998), we used the GTR+G substitution model for tree calculation using maximum likelihood for the unedited alignment and computed phylogenies based on the two manually edited alignments under the HKY+G model. We also constructed a ML tree under the T92+G (Tamura, 1992) model in MEGA 6

(Tamura et al., 2013) to account for uneven GC content in our sequences. We based bootstrap analyses for each tree on 1000 pseudo-replicates.

Ethical Note

We collected all gorilla samples non-invasively and with no harm to the study subjects. Permission to conduct research in the Dzanga-Sangha Protected Areas was granted by the Ministere de L'enseignement Supérieur et de la Recherche Scientifique and the Ministère des Eaux, Foréts, Chasses, Pêches, chargé de l'Environnement.

Results

Fungal diversity

We analyzed 238 clones with inserts of appropriate length from the clone libraries of amplified ITS1 fragments. The sequences we generated were associated with 45 different fungal rDNA sequences deposited in GenBank. Of the 238 clones we obtained, 78 were moderately similar to 12 different uncultured Neocallimastigales clones. These potential anaerobic fungi ITS1 fragments originated from 8 of 12 processed samples, with sequences similar to the Uncultured Neocallimastigales clone Iguana 01BMIEK (Acc. No. GQ843155) being the most abundant and the only one that occurs in all 8 samples. Other prospective anaerobic fungi ITS1 fragments that we amplified fit with uncultured Neocallimastigales clones detected in hindgut-fermenting Equidae, ruminant Bovidae, and the pseudo-ruminant hippopotamus (Table 1; Appendix S1).

The remaining fungal ITS1 fragments from gorillas that we cannot associate with anaerobic fungi clones are linked to sequences of the fungal classes Ascomycota and Basidiomycota (Appendix S1). These clones comprise 33 sequences that are related to 15 different strains of Ascomycota with moderate to high similarities (91 – 100%), covering five known orders and three strains of unclassified Ascomycota. Another four sequences that we obtained show high similarities (96 – 100%) with three

different Basidiomycota strains, belonging to three orders. According to BLAST analysis the majority of our ITS1 fragments are identified as unclassified fungal clones. In total, our sequences are related to 13 different such unclassified fungal clones that have been isolated from plant tissues, soil, reactor bio-filter, and woodpecker excavation with similarities ranging 96 – 100% (Appendix S1). An additional unclassified fungal clone (*Uncultured fungus clone AFI-1;* Acc. No. JX944983, unpublished sequence) to which 11 of our sequences are highly similar has been isolated from Bactrian camel (*Camelus bactrianus*) rumen. Finally, one sequence does not match with any of the rDNA sequences deposited in online data bases.

Phylogenetic analysis of anaerobic fungi

Our initial alignments revealed high degrees of resemblance among the potential anaerobic fungi sequences we obtained from gorilla feces, although they were associated with different uncultured Neocallimastigales clones (Table 1).

Table 1: Nearest relatives of ITS1 sequences retrieved from Western lowland gorilla feces collected at the sites Bai Hokou and Mongambe from September 2014 to January 2015.

UNC – Uncultured Neocallimastigales clone

* Not classified as Neocallimastigales fungus in NCBI (National Center for Biotechnology Information) sequence database.

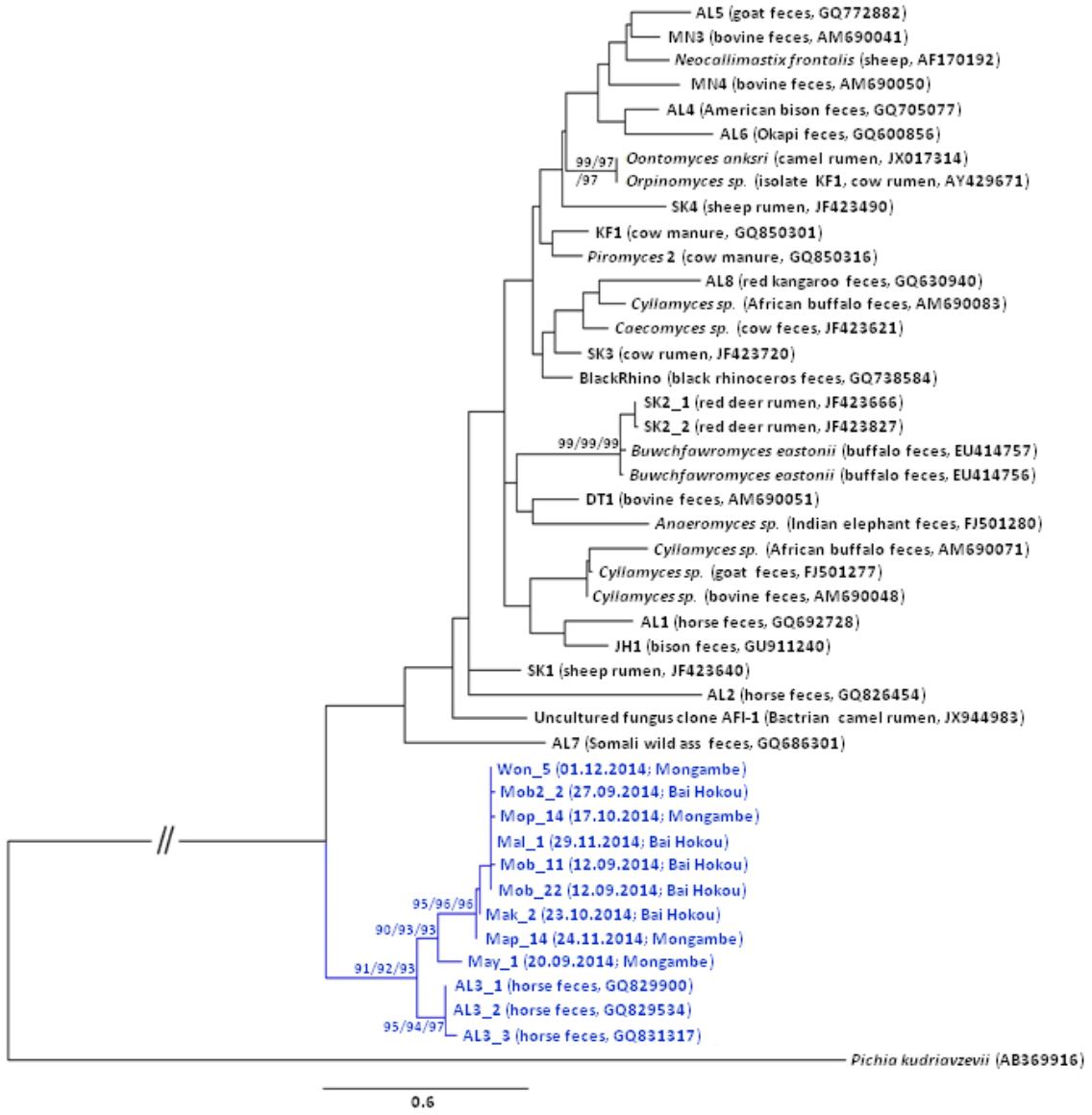
Sequence ID (date sample collection)	Field site	Size [bp]	GenBank Accession Number	Nearest relative [Accession Number]	Sequence similarity
Mak_2 (23.10.2014)	Bai Hokou	213	KY697108	UNC NileLechwe03FKYBS [GQ592255]	90
Mal_1 (29.11.2014)	Bai Hokou	283	KY697116	UNC HorseTopper01A6QWL [GQ688452]	89
Mob_11 (12.09.2014)	Bai Hokou	264	KY697114	UNC HorseBug01B20BM [GQ829356]	88
Mob_22 (12.09.2014)	Bai Hokou	279	KY697115	UNC Iguana01BLGEC [GQ843065]	88

Won_5 (01.12.2014)	Mongambe	260	KY697113	UNC Iguana01BMIEK [GQ843155]	90
May_19 (20.09.2014)	Mongambe	253	KY697112	UNC GrantsGazelle02CZ47B [GQ784902]	88
Mob2_2 (27.09.2014)	Bai Hokou	242	KY697109	UNC PigmyHippopotamus03GM37B [GQ607513]	89
Mop_14 (17.10.2014)	Mongambe	243	KY697110	UNC Iguana01A3GEE [GQ842869]	89
Map_14 (24.11.2014)	Mongambe	244	KY697111	Uncultured fungus clone AFI-1 [JX944983]*	100

In the Maximum Likelihood tree based on the original 452 bp alignment, our ITS1 fragments form a separate clade which clusters with the clade of the newly described uncultured anaerobic fungi group AL3 (group NG3 in Liggenstoffer et al., 2010) with significant support (Figure 1). This phylogenetic relationship is also supported in two other phylogenies that we constructed from 241 bp and 197 bp alignments. All other reference ITS1 sequences cluster in an unsupported monophyletic clade in which most of the phylogenetic relationships between the different groups and genera are rather weakly supported.

The Maximum Likelihood tree constructed under T92 + G substitution model, which accounts for uneven CG content in sequences, revealed very similar results for the sequence clustering. Again, fungal clones obtained from gorilla feces grouped with AL3 references with adequate support (bootstrap value 82; data not shown). However, as in the other three phylogenies, relationships between the reference sequences of known Neocallimastigales lack significant support.

Fig 1: Phylogenetic relationships of potential gorilla anaerobic fungi sequences in the order of Neocallimastigales fungi based on Maximum Likelihood. Bootstrap support above 50% is indicated at nodes for the 452, 241 and 197 bp alignments. Clones obtained in our study and reference sequences are listed in Table 1 and S2. Dates of sample collection and field site for sequences from gorilla samples are given in brackets.



245

246 **Discussion**

247 Our results suggest that anaerobic gut fungi are part of the gorilla gut microbiome. The assignment of
248 the ITS1 sequences we analyzed as a sister clade to the novel Neocallimastigales lineage AL3 is
249 significantly supported. Despite the highly significant support for the hypothesis that some of our
250 gorilla gut fungi belong to the class Neocallimastigomycetes, two factors warrant some caution. First,
251 fungal ITS1 sequences that we obtained from gorilla feces were only moderately similar to known
252 Neocallimastigales sequences deposited in the GenBank database. However, new lineages and
253 species of Neocallimastigales are constantly discovered (Ariyawansa et al., 2015; Hanafy et al., 2017).
254 Thus, our sequences might represent a new anaerobic fungi lineage. Second, our amplified ITS1

255 fragments were very short. This in combination with the known high variation in the
256 Neocallimastigales ITS1 region (Edwards et al., 2017) limits the reliability of constructed alignments
257 and phylogenies.
258
259 Like in other rapidly evolving non-coding regions insertions – deletions (indels) accumulate over time
260 in the ITS1 sequence. These indels are thought to be more conserved than base substitutions and
261 thus can provide a reliable source of information for phylogenetic reconstructions (Matheny et al.,
262 2006; Abarenkov et al., 2010). Alignment gap deletion decreases tree resolution, particularly when
263 sophisticated alignment algorithms such as MAFFT are applied (Nagy et al., 2012). Our phylogenetic
264 analysis based on alignment without gap removal significantly supports the close relationship of
265 gorilla gut fungi with the anaerobic fungi group AL3. Following the logic that alignment gaps can
266 provide phylogenetic information, this result supports our assignment of gorilla gut fungi to the class
267 of Neocallimastigales. The low bootstrap values in our phylogeny might be the result of difficulties
268 aligning anaerobic fungi sequences given the significant sequence dissimilarities and length
269 polymorphisms between genera (Nicholson et al., 2010). However, our goal was to determine
270 whether anaerobic fungi occur in wild gorillas rather than resolving the Neocallimastigales phylogeny.
271 Our sequences are very closely related to the anaerobic fungi group AL3. This group of
272 Neocallimastigales was first detected in hindgut fermenting equids which have similar digestive
273 physiology to gorillas. Since digestive physiology is a key factor determining anaerobic fungi
274 community structure (Liggenstoffer et al., 2010) it is likely that even distantly related herbivorous
275 animals harbor similar Neocallimastigales strains. This finding, therefore, provides additional support
276 for our hypothesis that Neocallimastigales are part of the gorilla gut microbiome.
277
278 While our analysis suggests that Neocallimastigales reside in the gastrointestinal tract of gorillas, we
279 have no indication so far that other African great apes harbor anaerobic fungi (unpublished data:
280 chimpanzee fecal samples, analyzed by D. Schulz). We predicted that anaerobic fungi are a part of the

gorilla gut microbiome based on gorilla diet and digestive physiology. Western lowland gorillas, although more frugivorous than mountain gorillas (*Gorilla gorilla beringei*), consume high fiber foods throughout the year (Rothman et al., 2008; Remis et al., 2001). The occurrence of anaerobic fungi in gorillas could therefore be interpreted as an adaptation to a high fiber diet. Along with other adaptive morphological and physiological digestive features (Harrison and Marshall, 2011) this might enable gorillas to survive on a low quality diet (Tutin et al., 1991). Other non-human primates that similarly rely on a highly or even strictly leafy diet could likewise benefit from harboring anaerobic fungi in their intestines. This remains to be investigated.

Gorillas fall back on more low-quality foods in periods of low preferred fruit abundance and in general consume much more fiber than chimpanzees (Wrangham et al, 1998, Tutin et al., 1991). Further, chimpanzees have smaller fiber digestions coefficients and their fecal microbial communities have diminished fiber degradation capacities compared to gorillas (Popovich et al., 1997; Conklin-Brittain et al., 2006; Kišidayová et al., 2009). Neocallimastigales play a pivotal role in digesting structural polysaccharides, particularly with regard to their ability to enhance access to fermentable substrate for hydrolyzing bacteria. Thus, the higher fiber degradation capacities of the gorilla gut microbiome might be the consequence of higher rates of bacterial fermentation facilitated by anaerobic fungi. However, given the limitations of sampling and methodology in our study, we draw this conclusion only cautiously.

Similar to the findings of a previous study (Hamad et al., 2014), we detected several Ascomycota and Basidiomycota strains in our gorilla samples. There is no concordance on the species level between Ascomycota strains we obtained and clones isolated by Hamad et al. (2014). However, four (Eurotiales, Hypocreales, Saccharomycetales, and Capnodiales) of six genera found by Hamad and colleagues are also present in our samples. Our results for Basidiomycota differ greatly from previously isolated strains in gorillas. While we isolated only four strains, it seems that the diversity of

Basidiomycota in the colonic fungal community of gorillas is actually far greater (Hamad et al., 2014). While some of the Basidiomycota strains detected in gorillas are human pathogens, a few of the identified Ascomycota, namely members of the order *Saccharomycetales* that are usually associated with plants, possess fermentative capacities (Hamad et al., 2014). It is, however, unclear whether these aerobic fungi constitute transients passing through the enteric tract with food particles or if they are residents and part of the gut microbiome with benefits for the host. We find the latter explanation unlikely due to the low redox potential of the anaerobic conditions in the intestinal tract (Espey, 2013).

In conclusion, our analyses provide evidence that Neocallimastigales is part of the gorilla gut microbiome. Our results emphasize the need to include enteric fungi when investigating the composition of the primate gut microbiome and we suggest that more research is needed to improve our understanding of the role of enteric fungi in the digestive tract. More extensive studies of fungal communities of several wild primate populations employing next generation sequencing techniques is warranted to enhance our knowledge of how differences in the fungal gut microbiome reflect differences in host diet and distribution. The results of such studies will contribute significantly to our understanding of the complexity of primate microbiomes and their adaptive values.

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Supplementary Material

Intestinal fungi strains identified and similarity with amplified ITS1 fragments (Appendix S1) and anaerobic fungi reference sequences (Appendix S2) and all sequences included in phylogenetic analysis (Appendix S3) are available online. If reasonable we will grant all further data requests from interested researchers.

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