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NOTES AND SHORT COMMUNICATIONS

Bacterial Diversity in the Cecum of the World's Largest Living Rodent (*Hydrochoerus hydrochaeris*)

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Abstract The capybara (*Hydrochoerus hydrochaeris*) is the world's largest living rodent. Native to South America, this hindgut fermenter is herbivorous and coprophagous and uses its enlarged cecum to digest dietary plant material. The microbiota of specialized hindgut fermenters has remained largely unexplored. The aim of this work was to describe the composition of the bacterial community in the fermenting cecum of wild capybaras. The analysis of bacterial communities in the capybara cecum is a first step towards the functional characterization of microbial fermentation in this model of hindgut fermentation. We sampled cecal contents from five wild adult capybaras (three males and two females) in the Venezuelan plains. DNA from cecal contents was extracted, the 16S rDNA was amplified, and the amplicons were hybridized onto a DNA microarray (G2 PhyloChip). We found 933 bacterial operational taxonomic units (OTUs) from 182 families in 21 bacterial phyla in the capybara cecum. The core bacterial microbiota (present in at least four animals) was represented by 575 OTUs. About 86% of the cecal bacterial OTUs belong to only five phyla, namely, Firmicutes (322 OTUs), Proteobacteria (301 OTUs), Bacteroidetes (76 OTUs), Actinobacteria (69 OTUs), and Sphirochaetes (37 OTUs). The capybara harbors a diverse bacterial community that includes lineages involved in fiber degradation and nitrogen fixation in other herbivorous animals.

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Introduction

The structural polymers of plant cell walls are the most abundant source of energy from primary producers, but vertebrate herbivores lack the enzymes necessary to digest these plant polymers. They can only nutritionally exploit them through symbiosis with microorganisms that can ferment structural carbohydrates into energy-rich byproducts such as short-chain fatty acids that provide energy for the host [26, 33]. Bacteria, protozoa, and fungi constitute the microbiota of the herbivore gut, where fermentative digestion depends on the efficiency of the gut microbiota activities and on the retention time of the digesta. Thus, the digestive tract of herbivores contain a voluminous fermentation chamber [21], and on the basis of the location of this chamber, herbivores can be divided into two groups: foregut and hindgut fermenters. While foregut fermenters degrade cellular soluble and structural plant carbohydrates into

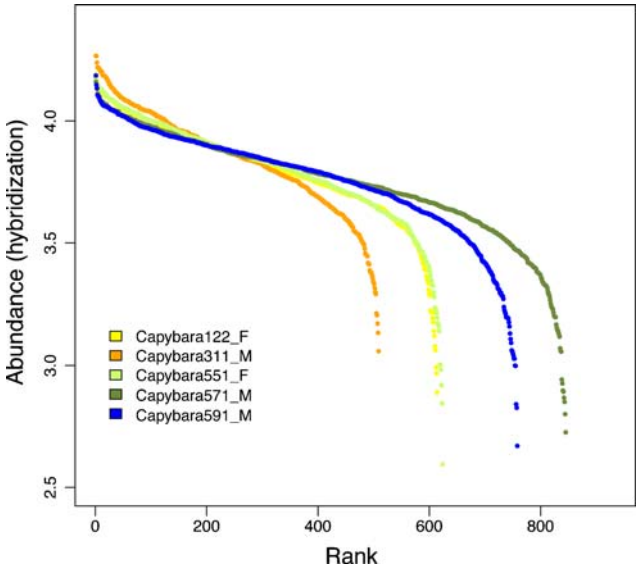

Figure 1 Rank–abundance curves of cecal bacterial communities for each capybara. Relative abundances were based on values of hybridization fluorescence in the PhyloChip

volatile fatty acids before they can reach the small intestine, hindgut fermenters degrade the remaining structural carbohydrates that bypassed digestion in the small intestine [1, 32].

In foregut fermenters, the fermentation chamber is anterior to the acid stomach, as in cows, camels, hippopotamuses, colobine monkeys, sloths, marsupials, and, in a single case in birds, the hoatzin [26]. In hindgut fermenters, the chamber is posterior to the acid stomach. Hindgut fermentation occurs either in the expanded colon of generally big mammals (horses, rhinoceros, elephants, tapirs, manatees, lagomorphs, howler monkeys) or in the cecum of generally small animals such as some arboreal marsupials and rodents [12, 32]. However, the biggest rodent, the capybara, *Hydrochoerus hydrochaeris* [32], is also a cecal fermenter.

Capybaras belong to the caviomorphs, a rodent group found only in South and Central America within the suborder Hystricomorpha [39]. They inhabit the seasonally flooded savannas and wetlands of Venezuela, Colombia, Brazil, Argentina, and other South American countries (except Chile). Adult capybaras are social animals, with little or no sexual dimorphism in size and a body weight of ~50 kg [10], being the largest living rodent and the largest known cecum-fermenting mammal [2]. They are grazers, with an exclusively herbivorous diet dominated by grasses [10]. They spend the early afternoon in the water, while in the evening and night they alternately graze and rest outside the water [15].

As with all caviomorphs, capybaras are coprophagous and re-ingest the special morning feces directly from the anus [15]. Coprophagy in capybaras is more frequent in the dry season, coinciding with the time of fewer food resources and highest cellulose content in the grass biomass. The capybara is adapted to grazing, with a fermenting cecum from which cultivable bacteria has previously been characterized [25]. The aim of the present study was to perform a molecular characterization of the bacterial microbiota in the capybara cecum.

Materials and Methods

Animals and Sampling

Five adult capybaras were captured at Hato Santa Luisa, a cattle ranch located in Apure State, Venezuela (8°19′ N, 70° 16′ W), in March 2009, during the dry season. The sampling took place during the capybara culling, carried out annually under a permit from the Venezuelan Ministry of the Environment, in which a large number of animals are hunted, mostly for their meat [24]. Immediately after the animals were killed (by gunshot), we sampled three males (identified as C311, C571, and C591) and two females (C122 and C551) by dissecting the gut and accessing the cecum. We placed 1.5 ml of cecal contents into a sterile microcentrifuge tube and immediately stored it in liquid nitrogen, to be later transferred to an ultralow temperature freezer until analysis.

DNA Extraction and Amplification

DNA was extracted from ~200 mg cecal contents from each animal, using the PowerSoil DNA Isolation Kit (MO BIO Inc., Carlsbad, CA, USA), and was kept frozen (−20°C) until use. Cecal DNA was PCR-amplified using 16S ribosomal primers 27F (5′-AGR GTT TGA TCM TGG CTC AG) and 1492R (5′-GGT TAC CTT GTT ACG ACT T), using eight different annealing temperatures (gradient from 48°C to 58°C, extension at 72°C). The PCR mix contained 25 μl of PCR Master Mix Cat No. M7505 (Promega, Madison, WI, USA) with ~50 ng of DNA template and 10 pmol of each primer using the same condition as described in [13].

Pooled products from each of the eight different annealing temperatures were purified using a PCR purification kit (Qiagen, Valencia, CA, USA).

PhyloChip DNA Hybridization

The 16S *rDNA* was hybridized onto the PhyloChip (Affymetrix, CA, USA) as previously described [3]. The G2 PhyloChip microarray has 506,944 probes representing ~8,700 bacterial and archaeal taxa [7]. The microarray has been validated and used to characterize bacterial commu-

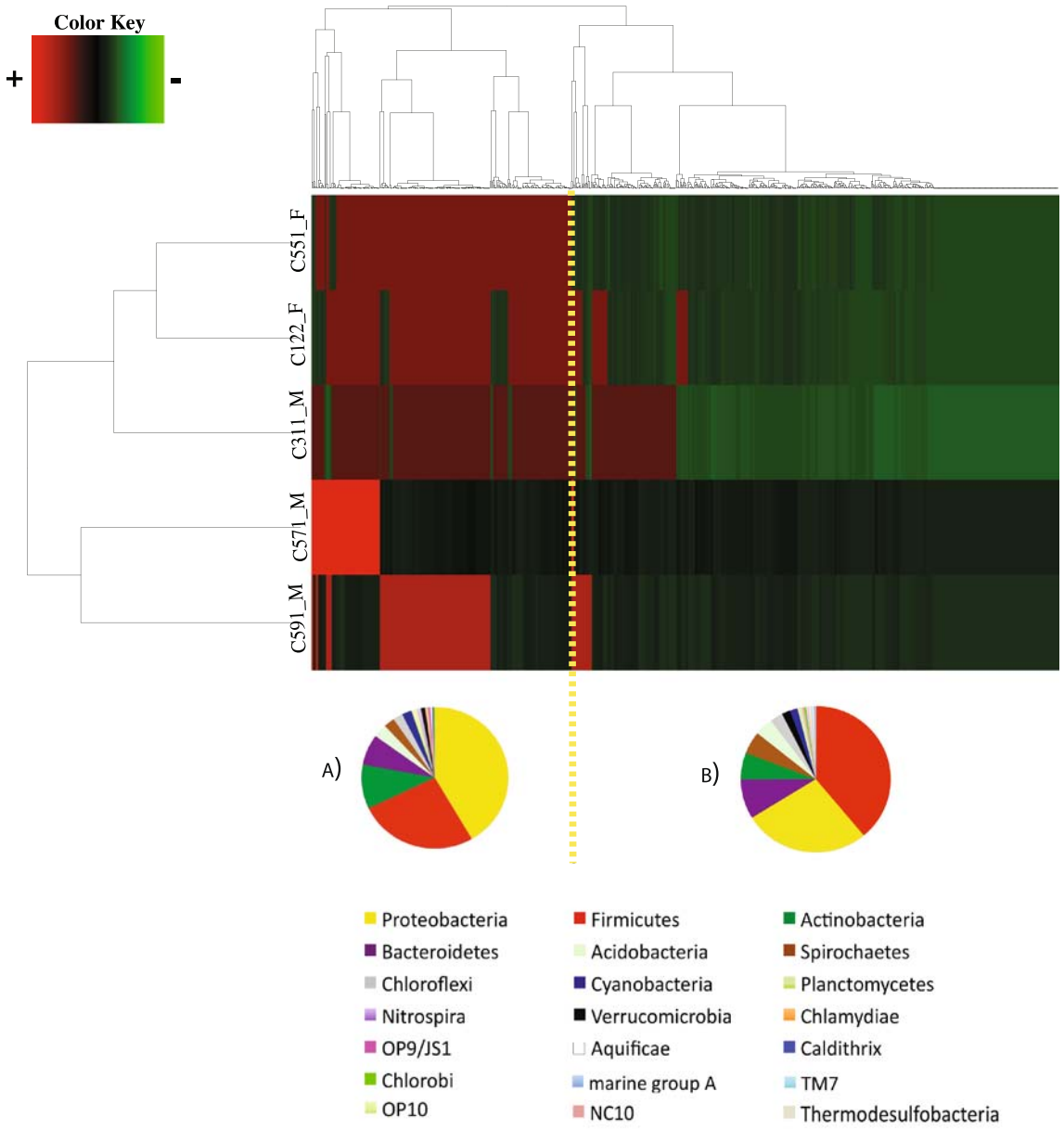

Figure 2 Heatmap and dendograms with bidirectional clustering of all 933 bacterial OTUs and specific host communities, showing the inter-host similarities. The color codes *red*/*green* indicate, respectively, a high/low in relative abundance for each OTU (shown here in *columns*). The *pie charts* on the *bottom* depict the phyla composition of the two main taxa clusters (here divided by a *dashed yellow line*): *A*—327 OTUs in 16 phyla that are in higher abundance in most samples and *B*—606 OTUs in 20 phyla that are less abundant, except for some OTUs in capybara male C311

nities [3, 7]. Its high sensitivity has been demonstrated, detecting 2.5-fold more diversity than cloning [4], although there are no species-level taxa obtained, as with Sanger sequencing. Instead, each operational taxonomic unit (OTU) is based on an average of 25 probe pairs, each consisting of a perfectly matched and a mismatched probe, and represents 16S rRNA gene sequences with 0–3% sequence divergence [7]. The sample amplicons were fragmented (to 50–200 bp) using DNase I (0.02 U/mg DNA; Invitrogen, USA) and One-Phor-All buffer (NJ, USA). Biotin labeling was performed with deoxyribonucleotransferase (Promega, USA). DNA was denatured at 99°C for 5 min and hybridization onto the PhyloChip was performed overnight at 48°C at 60 rpm. Scanning of the arrays was done using the GeneArray Scanner (Affymetrix, CA, USA) as previously reported [3, 13]. A taxon was reported present if at least 90% of the probe pairs in the set (probe fraction) had a perfect match probe with at least 130

Table 1 Numbers of operational taxonomic groups in 21 bacterial phyla from the cecal contents of five wild capybaras

Phylum	Class	Order	Family	Capybaras					Total
				C122	C551	C311	C571	C591	
Fimicutes	Clostridia	Clostridiales	Clostridiaceae	75	72	64	72	77	80
			Lachnospiraceae	62	62	54	68	60	70
			Peptostreptococcaceae	22	23	21	24	23	26
			Others	17	16	11	18	18	19
	Bacilli			37	41	33	87	39	91
	Others			26	29	27	31	30	36
Proteobacteria	Alphaproteobacteria	Bradyrhizobiales		11	10	8	31	23	31
		Rhizobiales		11	10	1	17	16	18
		Others		37	37	32	48	47	52
	Betaproteobacteria	Burkholderiales		5	7	2	17	19	23
		Others		2	1	0	12	6	14
	Deltaproteobacteria	Desulfobacterales		14	14	13	16	19	21
		Others		35	34	32	43	41	45
	Epsilonproteobacteria	Campylobacterales	Helicobacteraceae	17	17	18	18	24	24
			Others	10	10	9	10	13	13
	Gammaproteobacteria	Alteromonadales		4	5	1	12	6	12
		Others		20	23	11	38	33	48
Bacteroidetes	Bacteroidetes	Bacteroidales	Unclassified	14	13	13	19	18	19
			Others	20	19	16	22	22	23
	Others			25	25	17	29	28	34
Actinobacteria	Actinobacteria			31	38	30	61	50	69
Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	27	27	27	32	34	35
			Leptospiraceae	2	2	2	2	2	2
Others				90	89	67	118	110	128
Sum				614	624	509	845	758	933

times the square of background intensity and 1.3 times the mismatch probe intensity.

Data Analysis

To determine the common bacterial community composition, a core taxa (shared OTUs between at least four animals) was determined. Rank abundance curves were drawn from the data to visualize species richness and overall diversity using the vegan package in "R" http://www.R-project.org. A heatmap was calculated with hybridization fluorescence scores for the obtained OTUs using Pearson's correlation as the similarity metric and average linkage clustering in "R" using the package made4 [9].

In order to compare the individual cecal communities, community analyses were performed using FastUniFrac [14]. The community analyses are based on a clearcut tree [11, 31] generated from our data, an environment file with the PhyloChip-detected taxa for each individual, and a category mapping file consisting of a table with the sample names and its metadata. To determine the raw distances between bacterial communities in each pair of capybara cecal samples, we used the UniFrac metric (unweighted), resulting in a distance matrix where the smaller distances indicate the most similar pairs.

Principal coordinate analysis was performed unweighted to determine if the samples were distributed along any axes of variation that could be interpreted easily.

Results and Discussion

This work characterizes the composition and structure of the cecal community of the wild capybara. We found a total of 933 bacterial OTUs belonging to 182 families in 21 phyla. Figure 1 shows the individual rank abundance curves. The richness in the capybara cecum is lower than the ~1,400 OTUs observed in the hoatzin crop, also using the PhyloChip [13]. Consistent with previous studies of the human colon [28] or the hoatzin crop [13] microbiomes, the capybara cecal bacterial community has a high inter-

individual bacterial variation. Only half of the OTUs (464 out of 933 OTUs) were shared by all individuals. The individual variation was evident in the pattern and intensity of the different OTUs in Fig. 2, in which two males that cluster together (and share 74% of the OTUs) and one male and two females are together in a separate cluster (and share 69% of the OTUs). The clusters differ in the presence of Chlamydiae and phyla Thermodesulfobacteria, TM7, OP10, marine group A, and NC10. Beta-diversity analysis of the cecal bacterial communities using principal coordinate analysis shows the individual Unifrac distances (Fig. S1; Table S2), showing closer distances (higher similarity) between the communities of the two female individuals. However, the low number of animals does not allow one to draw any conclusion in relation to sex differences.

The majority of OTUs detected in the cecum of the capybara belonged to the Firmicutes (322 of 933 OTUs detected in the chip) and Proteobacteria (301 OTUs). The other phyla present in the capybara cecum were the Bacteroidetes (76 OTUs), the Actinobacteria (69 OTUs), and the Sphirochaetes (37 OTUs) (Table 1). The core bacterial microbiota was composed by 575 OTUs in 20 phyla (excluding Chlamydiae) with the same dominance of the Firmicutes (225 OTUs) and the Proteobacteria (155 OTUs), followed by Bacteroidetes, Actinobacteria, and Spirochaetes (Fig. 3). The phylum Firmicutes comprised 26 families with high numbers of taxa in the Clostridiaceae, Lachnospiraceae, and Peptostreptococcaceae (80, 70, and 26 OTUs, respectively) (Table 1). Within this phylum, the *Clostridium*, *Butyrivibrio*, and *Eubacterium* genera include known cellulolytic species commonly found in other mammalian intestines [6, 18, 19, 23], in rumen [17, 27], in the crop of hoatzins [13], as well as in the termite gut [16]. The detection of cellulolytic activity in the cecum of the capybara is consistent with the reported cellulolytic activity in cultures of isolated cecum contents [2].

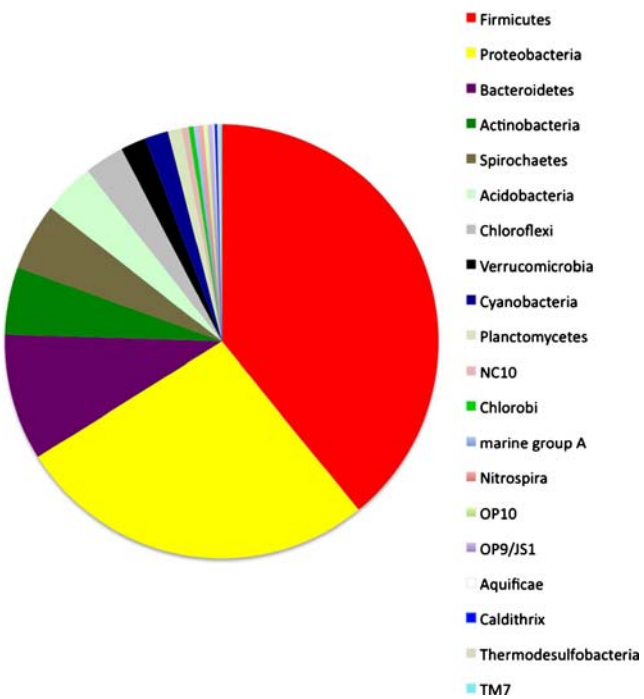

Figure 3 Phyla-level composition of the core microbiota in the Capybara cecum. The figure represents the richness of OTUs per phylum

There was high richness of the Proteobacteria in the cecum of the capybara. In our study, we only approximate the estimations of abundance through the intensity of the fluorescence of hybridized probes and cannot directly compare with the abundance estimations based on the sampling of bacterial DNA in Sanger or 454 sequencing procedures. This phylum is abundant in other mammals, ranking third (after Firmicutes and Bacteroidetes) in abundance among digestive bacteria in the hindgut of horses, pigs, rabbits, or humans [6, 18, 19, 23, 28]. In the capybara, the majority of cecal Proteobacteria OTUs belong to orders Bradyrhizobiales and Rhizobiales from Alphaproteobacteria class (Table 1). Proteobacteria in these groups are responsible for atmospheric nitrogen fixation in herbivorous ants and in rhizosphere of potato cultivars [29, 30, 34, 37]. It has been suggested that N_2 fixation by the Spirochetes phylum is important to termite nitrogen economy [20]. In mammals, bacterial nitrogen fixation has been reported in rodents consuming low-nitrogen diets (such as moles) as a mechanism for nitrogen supplementation [35]. In voles and in the European beaver, atmospheric nitrogen fixed in bacteria is utilized nutritionally via coprophagy [22, 36]. This might be the case of the capybara since grasses have low N_2 content and coprophagy may contribute to the capybara's nitrogen economy. However, future studies are needed to further explore the role of N_2-fixing species in the capybara nutrition.

Interestingly, Helicobacteraceae, a family commonly associated with gastrointestinal diseases in mammals [38], was one of the most predominant families from Epsilonproteobacteria in the capybara cecum (Table 1; Table S1), consistent with the report of the genus *Helicobacter* in the intestine of wild rodents [5, 8].

Among the less represented phyla, the Bacteroidetes included 76 OTUs with ~32% belonging to unclassified families (Table S1). Zoo capybaras seem to have a dominance of fecal Bacteroidetes (~60% of all sequences), according to Ley et al. [19], based on ~300 16S *rDNA* sequences. Our results are not directly comparable to Ley's due to differences in methodology, but differences in bacterial communities of the microbiome in wild and captive animals have been reported in mammals and birds [13, 19], highlighting the importance of pursuing studies in wild animals to understand the structure of un-impacted microbiotas.

The results presented here on the structure of the cecal bacterial communities in the capybara are an important first step towards a more comprehensive understanding of the digestive physiology of this large rodent. This is a pioneer but preliminary work, and the results lead to ecological questions and hypotheses testing that will benefit from using current sequencing technologies that are more robust and informative. Future studies monitoring the effects of temporal and spatial factors on the cecal bacterial communities using metagenomics will allow further understanding of community composition and function-informative genes involved in the digestion of cellulose and other substrates in the cecum of the capybara.

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References

1. Alexander RM (1993) The relative merits of foregut and hindgut fermentation. J Zool 231:391–401
2. Borges PA, DominguezBello MG, Herrera EA (1996) Digestive physiology of wild capybara. J Comp Physiol B 166:55–60
3. Brodie EL, DeSantis TZ, Joyner DC, Baek SM, Larsen JT, Andersen GL, Hazen TC, Richardson PM, Herman DJ, Tokunaga TK, Wan JMM, Firestone MK (2006) Application of a high-density oligonucleotide microarray approach to study bacterial population dynamics during uranium reduction and reoxidation. Appl Environ Microb 72:6288–6298
4. Brodie EL, DeSantis TZ, Parker JP, Zubietta IX, Piceno YM, Andersen GL (2007) Urban aerosols harbor diverse and dynamic bacterial populations. Proc Natl Acad Sci USA 104:299–304
5. Chichlowski M, Hale LP (2009) Effects of *Helicobacter* infection on research: the case for eradication of *Helicobacter* from rodent research colonies. Comp Med 59:10–17
6. Daly K, Stewart CS, Flint HJ, Shirazi-Beechey SP (2001) Bacterial diversity within the equine large intestine as revealed by molecular analysis of cloned 16S rRNA genes. FEMS Microbiol Ecol 38:141–151
7. DeSantis TZ, Brodie EL, Moberg JP, Zubieta IX, Piceno YM, Andersen GL (2007) High-density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. Microb Ecol 53:371–383
8. Dyson MC, Eaton KA, Chang C (2009) Helicobacter spp. in wild mice (Peromyscus leucopus) found in laboratory animal facilities. J Am Assoc Lab Anim Sci 48:754–756
9. Eisen MB, Spellman PT, Brown PO, Botstein D (1998) Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci USA 95:14863–14868
10. Escobar A, Gonzalez-Jimenez E (1974) Variación estacional de la frequencia relativa de las especies vegetales consumidas por los chiguires (*Hydrochoerus hydrochoeris*) en el llanos inundables. Acta Cient Venez 25:15
11. Evans J, Sheneman L, Foster J (2006) Relaxed neighbor joining: a fast distance-based phylogenetic tree construction method. J Mol Evol 62:785–792
12. Foley WJ, Cork SJ (1992) Use of fibrous diets by small herbivores—how far can the rules be bent. Trends Ecol Evol 7:159–162
13. Godoy-Vitorino F, Goldfarb KC, Brodie EL, Garcia-Amado MA, Michelangeli F, Dominguez-Bello MG (2010) Developmental microbial ecology of the crop of the folivorous hoatzin. ISME J 4:611–620
14. Hamady M, Lozupone C, Knight R (2010) Fast UniFrac: facilitating high-throughput phylogenetic analyses of microbial communities including analysis of pyrosequencing and PhyloChip data. ISME J 4:17–27
15. Herrera EA (1985) Coprophagy in the Capybara, *Hydrochoerus–Hydrochoeris*. J Zool 207:616–619
16. Hongoh Y, Ohkuma M, Kudo T (2003) Molecular analysis of bacterial microbiota in the gut of the termite *Reticulitermes speratus* (Isoptera; Rhinotermitidae). FEMS Microbiol Ecol 44:231–242
17. Koike S, Yoshitani S, Kobayashi Y, Tanaka K (2003) Phylogenetic analysis of fiber-associated rumen bacterial community and PCR detection of uncultured bacteria. FEMS Microbiol Lett 229:23–30
18. Leser TD, Amenuvor JZ, Jensen TK, Lindecrona RH, Boye M, Moller K (2002) Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. Appl Environ Microb 68:673–690
19. Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R, Gordon JI (2008) Evolution of mammals and their gut microbes. Science 320:1647–1651
20. Lilburn TC, Kim KS, Ostrom NE, Byzek KR, Leadbetter JR, Breznak JA (2001) Nitrogen fixation by symbiotic and free-living spirochetes. Science 292:2495–2498
21. Mackie RI (2002) Mutualistic fermentative digestion in the gastrointestinal tract: diversity and evolution. Int Comp Biol 42:319–326
22. Meshcherskii IG, Naumova EI, Kostina NV, Varshavslii AA, Umarov MM, YIur'eva OS (2004) Effect of deficiency of dietary nitrogen on cellulose digestibility and nitrogen-fixing flora activity in the sibling vole *Microtus rossiaemeridionalis*. Biol Bull 31:457–460
23. Monteils V, Cauquil L, Combes S, Godon JJ, Gidenne T (2008) Potential core species and satellite species in the bacterial community within the rabbit caecum. FEMS Microbiol Ecol 66:620–629
24. Ojasti J (1991) Chapter 17: Human exploitation of capybara. In: Robinson JG, Redford KH (eds) Neotropical wildlife use and conservation. Chicago University Press, Chicago, pp 236–252
25. Ojasti J (1973) Estudio biológico del chiguire o capibara Caracas: FONAIAP
26. Parra R (1978) Comparison of foregut and hindgut fermentation in herbivores. In: Montgomery G (ed) The ecology of arboreal folivores. Smithsonian Institution, Washington, pp 205–229
27. Pei CX, Liu Q, Dong CS, Hongquan L, Jiang JB, Gao WJ (2010) Diversity and abundance of the bacterial 16S rRNA gene sequences in forestomach of alpacas (*Lama pacos*) and sheep (*Ovis aries*). Anaerobe 16:426–432
28. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Jian M, Zhou Y, Li Y, Zhang X, Qin N, Yang H, Wang J, Brunak S, Dore J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Bork P, Ehrlich SD (2010)

A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464:59–65
29. Russell JA, Moreau CS, Goldman-Huertas B, Fujiwara M, Lohman DJ, Pierce NE (2009) Bacterial gut symbionts are tightly linked with the evolution of herbivory in ants. Proc Natl Acad Sci USA 106:21236–21241
30. Sawada H, Kuykendall LD, Young JM (2003) Changing concepts in the systematics of bacterial nitrogen-fixing legume symbionts. J Gen Appl Microbiol 49:155–179
31. Sheneman L, Evans J, Foster JA (2006) Clearcut: a fast implementation of relaxed neighbor joining. Bioinformatics 22:2823–2824
32. Stevens CE, Hume ID (eds) (1995) Comparative physiology of the vertebrate digestive system. Cambridge University Press, Cambridge
33. Stevens CE, Hume ID (1998) Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. Physiol Rev 78:393–427
34. Suen G, Scott JJ, Aylward FO, Adams SM, Tringe SG, Pinto-Tomas AA, Foster CE, Pauly M, Weimer PJ, Barry KW, Goodwin LA, Bouffard P, Li L, Osterberger J, Harkins TT, Slater SC, Donohue TJ, Currie CR (2010) An insect herbivore microbiome with high plant biomass-degrading capacity. PLoS Genet 6: e1001129. doi:10.1371/journal.pgen.1001129
35. Varshavskii AA, Puzachenko AY, Naumova EI, Kostina NV (2003) The enzymatic activity of the gastrointestinal tract microflora of the greater mole rat (*Spalax microphtalmus*, Spalacidae, Rodentia). Dokl Biol Sci 392:439–441
36. Vecherskii MV, Naumova EI, Kostina NV, Umarov MM (2009) Assimilation of biological nitrogen by European beaver. Biol Bull 36:92–95
37. Weinert N, Piceno Y, Ding GC, Meincke R, Heuer H, Berg G, Schloter M, Andersen G, Smalla K (2011) PhyloChip hybridization uncovered an enormous bacterial diversity in the rhizosphere of different potato cultivars: many common and few cultivar-dependent taxa. FEMS Microbiol Ecol 75:497–506
38. Whary MT, Fox JG (2004) Natural and experimental *Helicobacter* infections. Comp Med 54:128–158
39. Wilson DE, Reeder DN (2005) Mammal species of the world: a taxonomic and geographic reference. The Johns Hopkins University Press, Baltimore, p 2142