

1 **Ecology of Sleeping: The Microbial and Arthropod Associates of Chimpanzee Beds**

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3 Megan S. Thoemmes¹, Fiona A. Stewart^{2,3,4}, R. Adriana Hernandez-Aguilar^{2,5}, Matthew A.
4 Bertone⁶, David A. Baltzegar^{7,8}, Russell J. Borski⁷, Naomi Cohen², Kaitlin P. Coyle⁷, Alexander K.
5 Piel^{2,3}, Robert R. Dunn^{1,9}

6

7 1-Department of Applied Ecology and Keck Center for Behavioral Biology, North Carolina State
8 University, Raleigh, NC, USA

9 2-Ugalla Primate Project, Katavi Region, Tanzania

10 3-School of Natural Sciences and Psychology, Liverpool John Moores University, Liverpool, UK

11 4-Department of Archaeology and Anthropology, University of Cambridge, Cambridge, UK

12 5-Centre for Ecological and Evolutionary Synthesis, Department of Biosciences, University of
13 Oslo, Norway

14 6-Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC,
15 USA

16 7-Department of Biological Sciences, North Carolina State University, Raleigh, NC, USA

17 8-Genomic Sciences Laboratory, Office of Research, Innovation & Economic Development, North
18 Carolina State University, Raleigh, NC, USA

19 9-The Center for Macroecology, Ecology and Conservation, Museum of Natural History,
20 University of Copenhagen, Denmark

21 Corresponding author: msthoemm@ncsu.edu

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23

24 **Abstract**

25 The indoor environment created by the construction of homes and other buildings is often
26 considered to be uniquely different from other environments. It is composed of organisms that are
27 less diverse than those of the outdoors and strongly sourced by, or dependent upon, human bodies.
28 Yet, no one has ever compared the composition of species found in contemporary human homes to
29 that of other structures built by mammals, including those of non-human primates. Here we
30 consider the microbes and arthropods found in chimpanzee beds, relative to the surrounding
31 environment (n = 41 and 15 beds, respectively). Based on the study of human homes, we
32 hypothesized that the microbes found in chimpanzee beds would be less diverse than those on
33 nearby branches and leaves and that their beds would be primarily composed of body-associated
34 organisms. However, we found that differences between wet and dry seasons and elevation above
35 sea level explained nearly all of the observed variation in microbial diversity and community
36 structure. While we can identify the presence of a chimpanzee based on the assemblage of
37 bacteria, the dominant signal is that of environmental microbes. We found just four ectoparasitic
38 arthropod specimens, none of which appears to be specialized on chimpanzees or their structures.
39 These results suggest that the life to which chimpanzees are exposed while in their beds is
40 predominately the same as that of the surrounding environment.

41

42 **Introduction**

43 Humans modify landforms and build complex networks of structures in which we gather in
44 groups, store goods, and protect ourselves from harsh environmental conditions. Since the advent
45 of houses, which occurred between twenty thousand [1-4] and three hundred thousand years ago
46 [5], humans have become increasingly separated from the outdoor environment, and though there

47 is cultural variation in the design and use of buildings globally, human interactions with other
48 organisms now occur primarily within built structures [6]. It has been suggested that changes in the
49 types and diversity of species with which we interact, as a result of our shift indoors, have been to
50 our detriment, whether because we are no longer exposed to the diversity of environmental
51 bacteria necessary for our immune systems to fully develop (e.g., the hygiene hypothesis, [7]), or
52 because we fail to acquire commensal species on which our physical health and mental well-being
53 depend. A large body of literature [8-13], including a number of recent high profile books [14-17],
54 now considers the idea that these shifts in our interactions with other organisms are making us
55 sick. To varying extents, such work is predicated on the idea that our ancestors were exposed to
56 more and different kinds of microbes than we are currently, whether through various daily
57 activities or while they slept. Yet, no study has compared the species found in human homes, or
58 more generally in the modern built environment, to those found in structures built by other
59 mammals.

60 Many mammals sleep on the bare ground or in natural cavities, but a subset of mammals
61 construct modified structures in which to rest. The mammals that build these structures include
62 rodents and other taxa that dig burrows [18-19] and a smaller group of mammals, including some
63 primate species, that build modified aboveground sleeping places referred to, variously, as roosts,
64 nests or beds [20-21]. Great apes, including chimpanzees (*Pan troglodytes*), bonobos (*Pan*
65 *paniscus*), gorillas (*Gorilla* spp.) and orangutans (*Pongo* spp.), all build at least one bed a day to be
66 used for resting, before abandonment the following morning [22]. Due to the pervasiveness of this
67 behavior and the frequency of bed construction, it has been argued that these beds are the most
68 prevalent form of technology and material culture among extant great apes [23-24]. Although great

69 ape species differ in social organization, behavior and diet, all construct their beds in a similar
70 manner [22].

71 Chimpanzee beds, perhaps the best studied of the great ape beds, are complex structures
72 built by interweaving branches into a secure foundation covered by a leafy mattress. These beds
73 have been suggested to provide protection from the wind and other inclement weather, offer refuge
74 from predators, and increase comfort while resting. They are also hypothesized to reduce exposure
75 to pests and pathogens [21,24-31]. Chimpanzees spend over half their lives in beds, and they are
76 selective in the materials they use for construction, as well as to where they choose to build them
77 [32-35]. Because chimpanzees spend many hours in their beds each day, these structures are likely
78 to influence which species colonize the skin, guts and other habitats of chimpanzee bodies, and
79 their exposures to such groups are likely to have an impact on their immune systems.

80 Here we consider the bacteria and arthropods found in chimpanzee beds. More specifically,
81 we consider the diversity and likely origin of such species. Human homes are full of thousands of
82 species that slough off our bodies or consume dead skin, food waste and the house materials
83 themselves [36]. But it has been suggested that what is missing from many homes are the bacteria
84 and other organisms associated with soils, leaves and outdoor habitats [7,8]. Implicitly, this body
85 of research presumes that our ancestors were exposed to microbes and insects from diverse
86 environmental sources, including during the hours in which they slept. We might predict the same
87 for extant non-human great apes, such as chimpanzees. Alternatively, it may be that the overnight
88 contact of chimpanzees with their beds is sufficient to allow body-associated organisms to
89 accumulate, much as is the case for our own modern beds. To test these contrasting hypotheses, we
90 sampled chimpanzee beds in the Issa Valley, western Tanzania.

91 **Methods**

92 The Issa valley is situated within the Greater Mahale Ecosystem in Tanzania. It is more
93 than 90 km NE from the nearest national park boundary (Mahale Mountains), and roughly 60 km
94 SE from the nearest town (Uvinza). This region is characterized by broad valleys, separated by
95 steep mountains and flat plateaus, ranging from 900 – 1800 m above sea level. Vegetation is
96 dominated by miombo woodland - *Brachystegia* and *Julbernardia* (Fabaceae), interspersed with
97 swamp and grassland. A small proportion of the landscape (approximately 7%) is composed of
98 evergreen gallery and thicket riverine forests. There are two distinct seasons: wet (November –
99 April) and dry (May – October). Rainfall averages about 1200 mm per annum (range: 900 – 1400
100 mm, from 2001 – 2003; 2009 – 2014), and temperatures range from 11°C to 35°C [23,37]. The
101 core study area (85 km²) is used by one community of chimpanzees. As chimpanzees in Issa are
102 unhabituated to observers, the exact number of individual builders represented is unknown;
103 however, previous work by Rudicell et al. estimated this community to include approximately 67
104 individuals [38].

105 Within the study area, we collected microbes from chimpanzee beds (n = 41) and from
106 environmental locations (n = 41), as well as the arthropods associated with a subset of those beds
107 (n = 15 beds and 15 forest floor locations). Samples were collected between August 2013 and
108 April 2014. All chimpanzee beds were sampled following abandonment. Bed age was calculated
109 as time since construction and grouped into one of three classes; Fresh = 1 day, Recent = 2 - 7
110 days, and Old = 11 – 35 days (following Plumptre & Reynolds, [39]). Because the beds in our
111 study were not used for more than one night, time since abandonment and bed age are the same.
112 Additionally, though we know the identity of the chimpanzee community, we could not directly
113 observe which chimpanzee used a given bed; therefore, we do not consider how individual
114 variation influences the bacteria and arthropods present. We focus instead on the overall

115 differences in how organisms in chimpanzee beds vary relative to the natural habitat. Fieldwork
116 was approved by the Tanzanian Wildlife Research Institute (TAWRI) and the Commission for
117 Science and Technology (COSTECH); Permit No. 2014-202-ER-2011-94.

118 *Microbial Collection, Processing, and Analyses*

119 Dust samples to be used in microbial analyses were collected using dual-tipped sterile
120 BBL™ CultureSwabs™, identical to those used to study homes in the United States [36,40], as
121 well as the International Space Station [41]. We collected dust from two sample locations within
122 each chimpanzee bed; a branch used for bed construction (n = 41 beds) and, for a subset of beds, a
123 leaf that composed the mattress (n = 14 beds). As branches provide the structural support for
124 chimpanzee beds, we would expect frequent contact during building, general activity, and rest.
125 Additionally, we collected two environmental samples from within the same tree, at a height
126 similar to that of the sampled bed; a branch not incorporated into the bed (n = 41 locations) and a
127 leaf not incorporated into the mattress (n = 14 locations). These paired, environmental sites would
128 have presumably had much less exposure time, if any at all, to the chimpanzees. For our analyses,
129 we pooled branch and leaf samples and considered differences in surface type as a potential
130 explanatory factor in determining microbial diversity and community composition.

131 For each sample, we performed DNA extractions with a MO BIO PowerSoil® DNA
132 Isolation Kit (12888-100). Under sterile conditions, we removed one swab and swirled it against
133 the side of a PowerBead tube for 10 sec. We conducted all subsequent microbial DNA extraction
134 steps in accordance with the provided kit protocol, apart from step 19, in which we reduced the
135 quantity of Solution C6 to 50 µl to concentrate the eluted DNA. We then sent extracted DNA to
136 the Microbiome Core Facility, University of North Carolina Chapel Hill, School of Medicine
137 (USA) for PCR amplification and sequencing on the Illumina MiSeq platform. We targeted an

138 approximately 300 bp sequence, within the V1-V2 region of the 16S rRNA gene, with universal
139 primers: 8F 5'-AGAGTTTGATCCTGGCTCAG-3' and 338R 5'-GCTGCCTCCCGTAGGAGT-3'.

140 We merged overlapping reads with FLASH (v1.2.11, [42]), set to allow a maximum
141 overlap of 200 bp, and used the UPARSE pipeline (v8.0.1623, [43]) to cluster sequences into
142 operational taxonomic units (OTUs) at 97% similarity. We assigned taxonomy using the RDP
143 Classifier 2.2 in QIIME [44-45], trained on the Greengenes database (v13_8, [46]), and identified a
144 total of 8913 unique OTUs from 3,088,288 sequences. We removed low-quality or spurious OTUs
145 by applying several filters to the dataset. OTUs were removed if they had a merged consensus
146 sequence length outside the range of 310 to 370bp, if they had less than 50 total reads across all
147 samples, or if their taxonomy was flagged as cyanobacteria, mitochondria, or unassigned (15% of
148 total sequences; removed sequences in electronic supplementary material, table S1). The filtered
149 dataset contained 2,625,831 sequence reads over 1967 OTUs. We then rarefied those sequences to
150 5600 reads per sample and used the rarefied dataset for all downstream analyses. Of our 96
151 samples, four samples from within chimpanzee beds and four environmental samples did not meet
152 the minimum rarefaction threshold. We analyzed all data in the R environment with the *mctoolsr*
153 and *vegan* packages [47-49].

154 Using our rarefied dataset, we compared differences in OTU richness (measured by the
155 number of unique OTUs within a sample) and Shannon alpha diversity among samples with
156 Kruskal-Wallis tests. We tested the relative contribution of each potential explanatory factor on
157 both OTU richness and microbial community composition with permutational multivariate
158 analysis of variance (PERMANOVA), based on 999 permutations [50]. We quantified differences
159 among microbial communities through square-root transformation and the Bray-Curtis
160 dissimilarity metric and visualized community composition data with nonmetric multidimensional

161 scaling (NMDS) ordination plots. We included all potential explanatory variables of interest within
162 both the OTU richness and community composition PERMANOVA models, using an FDR
163 correction for multiple comparisons. Variables within these models included whether a sample was
164 from a chimpanzee bed, the age of a bed, season (wet or dry), elevation above sea level (m), and
165 whether a sample was from a branch or a leaf.

166 To assess the extent to which the microbial community within chimpanzee beds is
167 dominated by taxa from the same sources as those that are most abundant in human beds (i.e.,
168 fecal, skin, and oral associates; [36]), we used a source-tracking approach similar to those used
169 previously [36,51]. While the microbiota of humans and chimpanzees differ, a number of bacterial
170 taxonomic groups are characteristically associated with mammals [52,53], and an even larger
171 number is shared among great apes [54-56]. In order to determine whether a bacterial taxon is
172 likely to have come from the feces, skin or mouth of a chimpanzee, it would be ideal to
173 characterize the microbes from the wild chimpanzees within our study sites. However, since this
174 population of chimpanzees is unhabituated, we used body associate data from previous research.
175 We used data collected from wild and sanctuary primate populations within Africa to define a list
176 of bacterial taxa associated with chimpanzee feces and mouths (fecal: [57-59]; oral: [60];
177 supplementary table S2). Where data from wild chimpanzees were not available (i.e., skin
178 associates), we used taxonomic groups defined from the skin samples of captive chimpanzees [61]
179 augmented with bacterial taxa found by Ross to be ubiquitous across mammal orders, including
180 those of non-human primates ([52]; supplementary table S2). We do so while acknowledging that
181 some taxa common on the skin of wild chimpanzees might be missing in captive populations (as
182 seen in feces; [62-63]) and absent from other mammals. However, given the similarity of skin
183 microbiomes across mammal orders [52], we think this to be a reasonable starting point. We tested

184 all differences in the relative abundance of body-associated microbes between bed and
185 environmental samples with Kruskal-Wallis tests.

186 *Arthropod Collection and Analyses*

187 We collected arthropod specimens from 15 chimpanzee beds, at two locations per bed,
188 using a handheld insect vacuum (BioQuip products); inside the bed and the ground directly below
189 the bed (n = 30). We vacuumed each bed and ground location for two min. After collecting
190 samples, we stored them in 95% ethanol and shipped them to RR Dunn's lab (NC State
191 University) for specimen sorting and identification. MA Bertone identified arthropods to the
192 lowest possible taxonomic rank, based on morphology from intact specimens, in the NC State
193 Entomology and Plant Pathology lab. Due to the great diversity of poorly characterized
194 invertebrate species in Tanzania, particularly in the canopy [64], we were unable to identify many
195 of the specimens to species, or even family, level. However, because the arthropods associated
196 with primates have been well-studied [65], we were confident that we could identify such
197 specimens if present.

198 We calculated arthropod richness based on the identification of morphospecies and tested
199 differences in abundance between chimpanzee beds and the ground directly below each bed with a
200 Poisson distribution. We also assessed the likelihood of arthropods in the samples being
201 chimpanzee bed or human home associates and calculated the total number of known or potentially
202 blood-feeding ectoparasites based on biological information provided in the literature for the taxa
203 recovered [36,65]. Here we did not consider how arthropod communities vary with bed age. We
204 found so few ectoparasites that it was impossible to formally analyze differences among bed and
205 forest floor locations or to quantify changes over time, beyond reporting our raw counts and the
206 identification of each of the collected specimens.

207 **Results**

208 *Microbes*

209 We identified a total of 1896 microbial OTUs in chimpanzee beds and 1784 microbial
210 OTUs from environmental samples. Proteobacteria, Actinobacteria, and Bacteroidetes were the
211 most common phyla, accounting for 92.4% of sequence reads from beds and 91.4% of sequence
212 reads from environmental samples, with the phyla Proteobacteria and Actinobacteria accounting
213 for nearly all OTUs present. The most common families of bacteria in both the chimpanzee beds
214 and the surrounding environment were Methylocystaceae, Pseudonocardiaceae, and
215 Microbacteriaceae.

216 We observed no differences in the OTU richness or Shannon diversity of microbes in
217 chimpanzee beds, when compared to branches and leaves of the same tree (richness: $\chi^2 = 0.071$, p
218 $= 0.789$; average OTU richness per sample: bed = 343, tree branch or leaf = 357; Shannon
219 diversity: $\chi^2 = 1.288$, $p = 0.256$). When considering the relative contribution of all factors, season
220 was the strongest determinate of OTU richness across all samples. Whether samples were collected
221 in the wet or dry season accounted for nearly half of the observed variation ($R^2 = 0.43$, $p < 0.001$),
222 where richness was greatest during the dry season (figure 1). Elevation above sea level was the
223 next most explanatory variable ($R^2 = 0.31$, $p = 0.011$). When considering only the microbes found
224 in chimpanzee beds, age of the bed and whether samples were taken from branches or leaves did
225 not affect OTU richness ($p = 0.631$, $p = 0.811$, respectively; supplementary table S3a).

226 Just as with OTU richness, differences in community composition amongst all samples was
227 strongly influenced by season ($p < 0.001$) and elevation above sea level ($p < 0.001$). However,
228 here elevation explained 46% of the total observed variation, whereas season accounted for only
229 13% ($p < 0.001$). Within beds, the presence of one or more chimpanzees was a determinate of

230 microbial community composition, though the effect was small relative to the other factors ($R^2 =$
231 0.03, $p < 0.001$; supplementary figure S1). Bed age was not predictive of community assemblage
232 ($p = 0.714$; supplementary table S3b).

233 Of the top five most abundant bacterial genera known to be associated with chimpanzee
234 feces (as found in Yildirim et al., [58]), *Oscillabacter*, *Roseburia*, *Faecalibacterium*, and
235 *Caprococcus* were not found in any of our samples, regardless of whether the sample was
236 collected in or outside of a chimpanzee bed. Even closely related genera in the *Oscillabacter*
237 family, Oscillospiraceae, were not present. Fecal bacteria from the *Ruminococcus* genus were
238 present but rare (occurred in just 5% of samples and accounted for 0.008% of sequence reads) and
239 were no more abundant in beds than from environmental locations ($\chi^2 = 2.857$, $p = 0.090$). Even
240 when we expanded our dataset to include all fecal taxa [57-59]; supplementary table S2), we found
241 no difference in the proportion of fecal bacteria present in beds relative to branches or leaves of the
242 same tree ($\chi^2 = 1.649$, $p = 0.199$). Similar to the case for feces, skin-associated bacteria were no
243 more common in chimpanzee beds ($\chi^2 = 0.154$, $p = 0.695$; 2.4% of total reads) than in
244 environmental samples. Particularly noteworthy was that, although *Corynebacterium* is the most
245 abundant skin-associated taxonomic group currently described from chimpanzees (as well as from
246 gorillas) [61], we found no *Corynebacterium* in chimpanzee beds. Oral bacteria, on the other hand,
247 were more abundant in chimpanzee beds than on adjacent branches and leaves ($\chi^2 = 14.644$, $p <$
248 0.001). However, these too represented a very small portion of the total abundance of all microbes
249 (0.82% of sequence reads from beds, 0.03% of sequence reads from the environment).

250 Collectively, body-associated taxa (be they fecal, skin or oral in origin) accounted for only 3.5% of
251 all microbial sequence reads from within chimpanzee beds.

252 *Arthropods*

253 Arthropods were more abundant on the ground than in chimpanzee beds ($p = 0.007$; $n =$
254 226 ground specimens, $n = 108$ bed specimens; table 1). Nonetheless, beds ($n = 15$) were host to
255 12 orders of arthropods, comprised of 47 total morphospecies, with an average of 5.2 orders and
256 3.1 morphospecies represented per individual bed. Of all morphospecies collected just two are
257 known ectoparasites of mammals (Phlebotominae and Ceratopogonidae, $n = 3$). All three
258 specimens from these families were collected from within beds. We also collected one specimen of
259 a potential blood-feeder from the Anthocoridae family ($n = 1$; table 1). We collected one
260 Ceratopogonidae larva from the ground below a chimpanzee bed; however, though the adults of
261 Ceratopogonidae are blood-feeders, the larvae are not, so this specimen was not included in the
262 total number of ectoparasites.

263 Of all arthropods collected within beds, none was from a lineage known to be strongly
264 dependent on chimpanzees or mammal structures [65,66]. One potential exception was that of the
265 silvanid beetles (Silvanidae). These beetles are often found in human homes [66]; however, after
266 further identification, we found that the silvanid beetles collected from chimpanzee beds belonged
267 to the genus *Airaphilus*. The beetles within this genus feed on fungal spores and dead plant
268 material and are commonly found beneath the bark of dead trees or in leaf litter. Due to their
269 ecological niche, it is unlikely to be a group directly associated with chimpanzee bodies or
270 structures ([67], personal communication Dr. Michael C Thomas).

271 **Discussion**

272 The exposure of a mammal to pathogens, environmental bacteria, insects and other
273 sympatric taxa is likely to be strongly influenced by the ecology of its sleeping place. We
274 hypothesize that this has been the case for tens of millions of years, such that mammalian immune
275 systems have evolved in the context of frequent exposure to environmental microbes. It has

276 become increasingly clear that which species mammals, including humans, are exposed to can
277 have both beneficial and detrimental effects on health and well-being. It has often been suggested
278 that we have reduced the diversity of our exposures to other species, as we have begun to spend
279 more time indoors. Yet, little is known about what those interactions might have been historically,
280 or how such interactions vary among our living relatives. Here we present the first study of the
281 organisms found in the sleeping place of a non-human mammal, that of wild chimpanzees.

282 Based on the study of human homes [36], one might hypothesize that the microbes found in
283 chimpanzee beds would be less diverse than that of the adjacent environment, and further, that
284 chimpanzee beds would be primarily composed of body associates. Instead, we found that the
285 diversity of bacteria in chimpanzee beds was similar to that of the surrounding environment
286 (supplementary table S3a). In addition, taxa from chimpanzee bodies were almost entirely lacking
287 in beds. Though we recognize that there is still more research needed on the characterization of
288 microbiomes from wild chimpanzees, the near complete absence of currently defined body-
289 associated taxonomic groups from within chimpanzee beds indicates that there is likely to be little
290 accumulation of such species. The construction and likely inhabitation of a bed influenced which
291 bacteria were present; however, the season in which each bed was built and the elevation above
292 sea level explained most of the variation in microbial diversity and community assemblage,
293 respectively (supplementary table S3). Similarly, we found only four arthropod individuals known
294 to be ectoparasites within beds, none of which appears to be a specialist on chimpanzees or their
295 structures (table 1). In short, our results suggest that the microbes and arthropods to which
296 chimpanzees are exposed while resting are predominately environmental, contingent upon season
297 and location on the landscape.

298 The beds made by great apes, be they chimpanzees, gorillas, bonobos or orangutans, are
299 typically used for a single night and then abandoned [22]. This movement of beds from one night
300 to the next has long been thought to serve a range of beneficial functions. One explanation for such
301 movement is that it decreases the ability of pathogens and pests to build up at a sleeping site and
302 reduces the microbial odors associated with the individual that might attract predators [68-69]. Our
303 results are commensurate with this hypothesis, as we found little evidence of the accumulation of
304 bacteria or arthropods in chimpanzee beds. The lack of fecal bacteria may also be due to
305 chimpanzee toilette hygiene. Chimpanzees usually defecate over the sides of their beds [70]. Our
306 data suggest they are effective at doing so in a way that prevents soiling the beds themselves. In
307 addition, we found no arthropods in beds that were closely associated with chimpanzees and only
308 four mobile blood-feeder specimens. Yet, chimpanzees are host to more than 60 parasites and
309 pathogens, including lice and fur mites [65,71-72]. Given this, our results may reflect effective
310 grooming practices (such as consuming ectoparasites), which prevent those species from reaching
311 high abundances even when present. These findings highlight the need for more research on wild,
312 habituated primate populations which would allow for the direct collection of microbes and
313 arthropods from individuals and access to beds immediately following abandonment. We could
314 then more fully explore the strength of individual variation, as well as directly observe behavior
315 within beds, which was not possible within the scope of our study.

316 *Invention of the Indoors*

317 Though chimpanzees are not human ancestors, having diverged from a common ancestor
318 between 6.6 and 12 million years ago [73-74], the building of beds by great apes is an ancestral
319 trait that is thought to have appeared before the divergence of the hominid and hominin lineages
320 [21-22,24]. Chimpanzees have often served as a model for reconstructing the behavior of early

321 hominin species [75-79], including the evolution of structure building [24]. Furthermore, it has
322 been hypothesized that early hominins built beds in which to rest, as is seen among modern great
323 apes [79-82]. Based on the reconstructed history of building among these groups, the beds of
324 chimpanzees are likely to share common features with those of our hominin ancestors, especially
325 given that our ancestors exhibited morphological adaptations for arboreality (*Ardipithecus*
326 *ramidus*, [83]; *Australopithecus afarensis*, [84]; *Homo habilis*, [85]) and may have moved from
327 sleeping site to sleeping site, as has been argued [37,81]. In as much, chimpanzee beds offer a
328 window into the potential exposures of our ancestors while sleeping, even if an imperfect one.

329 Chimpanzee beds and human homes share two of the three most abundant microbial phyla
330 (Proteobacteria and Actinobacteria). However, this similarity hides major differences in the likely
331 origins of these microbes, differences that can be better seen if we consider the taxonomic level of
332 families. Methylocystaceae, Pseudonocardiaceae, and Microbacteriaceae were common in
333 chimpanzee beds and are all previously described environmental microbes and/or soil associates
334 [86-88]. In contrast, the most abundant families of bacteria in human homes are those associated
335 with human skin or feces; Streptococcaceae, Corynebacteriaceae, and Lactobacillaceae [36]. To
336 put it simply, we have created sleeping places in which our exposure to soil and other
337 environmental microbes has all but disappeared, and we are instead surrounded by less diverse
338 microbes that are primarily sourced from our own bodies [36,89]. The situation is similar with
339 regard to arthropods. Chimpanzee beds contained no arthropod specimens specialized on life with
340 chimpanzees. In contrast, the arthropod communities in human homes are diverse, often including
341 hundreds of species, tens of which are specialized on life indoors with humans [6,66].

342 We do not yet know enough to reconstruct the complete history of human sleeping places
343 and the species that composed their communities. However, we can propose based on our results

344 from chimpanzee beds that at some point in hominin evolution, likely no earlier than a million
345 years ago [90-91] and no later than twenty thousand years ago [1-2], our ancestors made a major
346 transition in terms of their exposures to other organisms while sleeping. They began to sleep
347 repeatedly in the same spots and, in doing so, provided the opportunity for recurrent exposures to
348 the subset of species that live on bodies and in beds and homes. With that change, the proportion
349 of time we spend with these species has continued to increase with the proportion of time we
350 spend indoors. Meanwhile, our exposure to environmental microbes and arthropods has decreased.
351 If true, exposure to our own microbes and to the arthropods adapted to the human built
352 environment may be novel, relative not only to our recent history but also potentially to our more
353 ancient past.

354

355 **Ethics Statement**

356 No data were collected from human or animal subjects for the purpose of this study. All
357 chimpanzee beds were sampled following site abandonment, and there was no direct contact with
358 any of the chimpanzee individuals.

359 **Data Accessibility**

360 Microbial data from this project were deposited in the Dryad data repository and made publically
361 available at doi:10.5061/dryad.7cp50

362 **Competing Interests**

363 We have no competing interests to declare.

364 **Authors' Contributions**

365 MST designed collection protocols, coordinated sample processing, and conducted statistical
366 analyses; FS, RAHA, & NC coordinated and conducted field data collection; AKP facilitated data

367 collection and permitting; RRD conceived of and FS, RAHA, & RRD designed and coordinated
368 this research project, MB identified all arthropod specimens; DAB designed protocols and
369 conducted extractions for microbial samples; RB contributed resources and lab space; KPC
370 processed microbial sequencing data and aided in the interpretation of results. Manuscript was
371 drafted by MST, FS, RAHA, AKP, MB, DAB, KPC, and RRD. All authors gave final approval for
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384 **References**

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698 **Figure and Table Captions**

699 Figure 1. The OTU richness among all samples was primarily driven by differences in wet and dry
700 seasons ($p < 0.001$). Season accounted for approximately 43% of the observed variation, with no
701 difference between chimpanzee beds and the environment ($p = 0.509$). OTU richness was greatest

702 in the dry season overall, as well as when chimpanzee beds or environmental samples were
703 considered on their own ($R^2 = 0.54$, $p < 0.001$; $R^2 = 0.32$, $p < 0.001$, respectively).

704

705 Table 1. Arthropod specimens. Specimens were identified to the family or group level.

706 Presence/absence data were noted for chimpanzee bed and ground samples. All specimens
707 indicated as parasites are from taxa that include ectoparasites.

708

709 Figure S1. Nonmetric multidimensional scaling (NMDS) ordination plot. NMDS plot representing
710 the overall variation in microbial community composition as a function of sample type. Sites are
711 coded based on whether they were collected from within a chimpanzee bed or from environmental
712 samples (leaves and branches of the same tree). Note a differentiation between a subset of beds
713 (bottom left) and the environment ($p < 0.001$).

714

715 Table S1. OTUs removed prior to rarefaction and analyses. All removed OTUs were spurious, of
716 low quality, or designated as unassigned, mitochondrial, or chloroplast sequences. To provide
717 support for the removal of any OTUs with greater than 1000 reads across all samples, NCBI blast
718 results were reported.

719

720 Table S2. Chimpanzee body-associated bacterial taxa used in source-tracking analyses.

721

722 Table S3. PERMANOVA results. Data were analyzed following rarefaction. All potential
723 explanatory variables were included within both the OTU richness and community composition
724 PERMANOVA models, using an FDR correction for multiple comparisons. Variables included

725 whether a sample was collected from a chimpanzee bed, the age of a bed, season (wet or dry),
726 elevation above sea level (m), and whether a sample was from a branch or a leaf. (a) Alpha
727 richness values were calculated based on the number of unique microbial OTUs present in each
728 sample. (b) Community composition data were weighted by OTU abundance using the Bray-Curtis
729 dissimilarity metric.