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1

2 **Microbiome variability of mosquito lines is consistent over time and across**

3 **environments**

4

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19 **Abstract**

20 The composition of the microbiome is shaped by both environment and host genetic
21 background in most organisms, but in the mosquito *Aedes aegypti* the role of host genetics in
22 shaping the microbiome is poorly understood. Previously, we had shown that four lines of *Ae.*
23 *aegypti* harbored different microbiomes when reared in the same insectary under identical
24 conditions. To determine whether these lines differed from each other across time and in
25 different environments, we characterized the microbiome of the same four lines of *Ae. aegypti*
26 reared in the original insectary and at another institution. While it was clear that the environment
27 influenced the microbiomes of these lines, we did still observe distinct differences in the
28 microbiome between lines within each insectary. Clear differences were observed in alpha
29 diversity, beta diversity, and abundance of specific bacterial taxa. To determine if the line
30 specific differences in the microbiome were maintained across environments, pair-wise
31 differential abundances of taxa was compared between insectaries. Lines were most similar to
32 other lines from the same insectary than to the same line reared in a different insectary.
33 Additionally, relatively few differentially abundant taxa identified between pairs of lines were
34 shared across insectaries, indicating that line specific properties of the microbiome are not
35 conserved across environments, or that there were distinct microbiota within each insectary.
36 Overall, these results demonstrate that mosquito line can shape the microbiome across
37 microbially- diverse environments and host by microbe interactions affecting microbiome
38 composition and abundance is dependent on environmentally available bacteria.
39

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40 **Author Summary**

41 The mosquito microbiome plays a critical role in shaping interactions with human pathogens.
42 The factors that contribute to shaping the composition of the mosquito microbiome are of high
43 importance due to its role in pathogen interactions and the successful development of control
44 strategies. In other organisms, both host genetics and environment shape the microbiome
45 composition, but the role of host genetics in shaping the mosquito microbiome is less clear.
46 Previously, we have shown that different lines of *Aedes aegypti* harbor different microbiomes
47 when reared in the same environment. We were curious to see if these differences could still be
48 detected after further generations in the same insectary and across environments in a different
49 insectary. We found that found that the microbiome differed between these lines in each
50 insectary indicating an element of both host genetic background and environment play a role in
51 establishing the microbiome. Our results indicate that different genetic backgrounds of *Ae.*
52 *aegypti* will interact with their environment differently to shape their microbiome, which could
53 potentially influence interactions with human pathogens and/or the effectiveness of control
54 strategies. More broadly, our results are of interest for the ecology of host-microbe interactions.
55

56 **Introduction**

57 The microbiome plays a crucial role in the health of various organisms [1, 2]. Alterations in the
58 relative abundance and overall bacterial community structure can lead to dysbiosis within the
59 organism resulting in disease or morbidity. Factors that shape the acquisition and maintenance
60 of the microbiome vary between organisms and include contributions from both the environment
61 and the host. The role of host genotype in shaping the composition of the microbiome has
62 become apparent in mammalian systems where specific host genomic loci have been
63 associated with specific bacterial taxa [3-5]. Additionally, the role of host genetic background in
64 invertebrates such as *Drosophila* and other insects has also been shown to contribute to the
65 composition of the microbiome [6-9]. While the contribution of host genetic background in

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66 shaping the microbiome is well studied in mammalian and *Drosophila* systems, few and
67 contradictory data exist for the role of host genetic background in shaping the microbiome of the
68 mosquito [10-12].
69
70 *Aedes aegypti* is the main vector of arthropod-borne viruses (arboviruses) worldwide, such as
71 dengue, Zika, and chikungunya. Examples such as dengue virus (DENV) result in 100-400
72 million infections annually and remain a major threat to public health [13, 14]. The microbiome,
73 specifically the presence of distinct isolates, plays a role in the ability of *Ae. aegypti* to be a
74 successful vector of human pathogens [15, 16]. The microbiome of *Ae. aegypti* is largely
75 shaped by the environment [17, 18], but the role of host genetic background remains poorly
76 understood. Multiple studies have controlled for environmental variation and characterized the
77 microbiome of diverse lines of *Ae. aegypti* reared in the same insectary (i.e. the same
78 environment). These studies have found contradictory results. Two independent studies
79 identified differences in the microbiome from the whole body of *Ae. aegypti* that were dependent
80 on mosquito line [10, 11]. A third study did not observe any differences in either the bacterial
81 community structure or the abundance of specific taxa in the midgut from a selection of
82 genetically diverse lines of *Ae. aegypti*, which represented their worldwide genetic diversity [12].
83 These contradictory results suggest that perhaps the environment is important for detecting line
84 specific differences in the microbiome, or alternatively, there are host and environmental
85 interactions that determine the microbiome composition in *Ae. aegypti*.
86
87 *Aedes aegypti* occupies a variety of environments worldwide, allowing for the association of
88 numerous bacterial taxa with it. Understanding the relative contributions of *Ae. aegypti* genetic
89 background and the environment in shaping the microbiome composition is important for both
90 teasing apart the role of the microbiome in mosquito vectorial capacity, [15] as well as
91 development of paratransgenic control tools [19-21]. To understand whether line specific

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92 differences in the microbiome is dependent on the environment, we sequenced the 16s
93 ribosomal RNA gene from four lines of *Ae. aegypti* that previously showed line specific
94 differences in the microbiome [10] and the same four lines after being transferred to a new
95 insectary at another institution. We analyzed the structure of the bacterial community between
96 and within each insectary, as well as the differential abundance of specific taxa. Finally, we
97 identify conserved genera that differ in pairwise comparison between lines at each insectary.
98 Our findings that lines harbor differences in their bacterial composition despite being reared
99 under identical conditions has important implications when comparing phenotypic effects which
100 may be sensitive to the microbiome.

101 <u>**Materials and Methods**</u>

102 ***Ae. aegypti* Mosquitoes**

103 Colonies of *Ae. aegypti* used in this study originated from Galveston, Iquitos, Juchitan, and
104 Thailand. At UTMB, the generation of the colonies is not known but the colonies have been in
105 the insectary since 2010 and reared continuously. The colonies were transferred to LSTM in
106 2018. At each institute the mosquito lines were housed under standard insectary conditions
107 consisting of 28°C and 70% +/- 10% relative humidity with 12h:12h light dark cycle. Eggs were
108 hatched in deionized water and larvae were fed fish food. Adults were held in Bugdorm cages
109 with constant access to 10% sucrose until being harvested.
110

111 **DNA Extractions**

112 Three-five days post emergence, *Ae. aegypti* were cold anesthetized and females taken for
113 surface sterilization. Individual mosquitoes were surface sterilized in ethanol 70% for 5 minutes
114 followed by 3 washes in sterile PBS. DNA was extracted from 20 female mosquitoes from
115 Galveston, Iquitos, Juchitan and Thailand using the QIAamp DNA Mini Kit (QIAGEN) following
116 the manufacturer protocol with the following modifications: initial volume of 180ul of buffer ATL
117 used for mosquito homogenization and final volume of 100ul nuclease-free water for DNA

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118 elution. No RNase A treatment was applied. For the UTMB samples, DNA from mosquitoes
119 from Iquitos and Juchitan was extracted on 25 February 2021 and DNA from mosquitoes from
120 Galveston and Thailand was extracted on 26 February 2021. No-mosquito controls were used
121 for each extraction batch and sequenced. The negative control clustered differently from the
122 samples (Supplemental Figure 1), with the exception of Juchitan.
123

124 **Library Preparation and Sequencing**

125 Sequencing libraries for each isolate were generated using universal 16S rRNA V3-V4 region
126 primers [22] in accordance with Illumina 16S rRNA metagenomic sequencing library protocols.
127 DNA concentrations of each library were determined by Qubit and equal amounts of DNA from
128 each barcoded library were pooled prior to sequencing. The samples were barcoded for
129 multiplexing using Nextera XT Index Kit v2. The pooled libraries were diluted to 4 pM and run on
130 the Illumina Miseq using a MiSeq Reagent Kit v2 (500-cycles).
131

132 **Data Analysis**

133 To identify known bacteria, sequences were analyzed using the CLC Genomics Workbench
134 21.0.5 Microbial Genomics Module (CLC MGM). Reads containing nucleotides below the quality
135 threshold of 0.05 (using the modified Richard Mott algorithm) and those with two or more
136 unknown nucleotides or sequencing adapters were trimmed out. Reference-based Operational
137 Taxonomic Unit (OTU) picking was performed using the SILVA SSU v132 97% database [23].
138 Sequences present in more than one copy but not clustered to the database were placed into
139 de novo OTUs (97% similarity) and aligned against the reference database with an 80%
140 similarity threshold to assign the "closest" taxonomical name where possible. Chimeras were
141 removed from the dataset if the absolute crossover cost was three using a k-mer size of six.
142 OTUs with a combined abundance of less than two were removed from the analysis. Low
143 abundance OTUs were removed from the analysis if their combined abundance was below 10

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144 or 0.1% of reads. Differential abundance analysis was performed using CLC MGM at the genus
145 level to compare the differences between the groups using trimmed mean of M-values. Each
146 OTU was modeled as a separate generalized linear model, where it is assumed that
147 abundances follow a negative binomial distribution. The Wald test was used to determine
148 significance between groups. Tables of differentially abundant taxa are on the complete
149 unfiltered data set (Supplemental Tables 1 and 2). To perform the analyses of the number of
150 genera differentially abundant shared between various pairwise comparisons, the ID of the
151 genera from the filtered OTU table were pulled out of the full data file using the “join” command
152 in R. Only the ID of genera from the filtered OTU table were used.
153
154 Abundance profiling was performed using MicrobiomeAnalyst [24, 25]. The analysis parameters
155 were set so that OTUs had to have a count of at least 10 in 20% of the samples and above 10%
156 inter-quantile range. Analysis was performed using actual and total sum scale abundances.
157 Alpha diversity was measured using the observed features to identify the community richness
158 using Chao1. Statistical significance was calculated using T-test/ANOVA. Beta diversity was
159 calculated using the Bray-Curtis dissimilarity measure (genus level). Permutational Multivariate
160 Analysis of Variance (PERMANOVA) analysis was used to measure effect size and significance
161 on beta diversity for grouping variables [26]. Relative abundance analysis was done in
162 MicrobiomeAnalyst at the level of genera.
163
164 **Upset plot**
165 To identify the distribution of bacteria taxa between the different mosquito lines and their rearing
166 insectaries, count data from female mosquitoes were extracted and aggregated to the genus
167 level for each sample. This yielded a maximum of 44 distinct bacterial taxa (including
168 'uncultured' and 'ambiguous' taxa). This table of count data was then processed using the R
169 package UpsetR [27], stratifying the data based on mosquito strain and the rearing insectary.

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170

171 **Correlation plot**

172 Previous studies have indicated a potential correlation in presence/absence between specific
173 genera of bacteria- namely *Cedecea*, *Enterobacter*, *Klebsiella*, and *Serratia*. To test for this,
174 counts from the four genera were extracted from the aggregated table described earlier. Using
175 the sum of these counts as the total, percentage relative abundance for each taxa was then
176 counted per individual sample. These results were then passed to the cor() function in R's stats
177 package [28] to obtain a correlation matrix of size four-by-four. This correlation matrix was then
178 visualised using the R package corrplot [29], with the two options method = "color", col =
179 COL2('PiYG')**.**

180

181

182 **Results**

183 **Between Insectary Differences**

184 We previously reported that the microbiomes of four *Ae. aegypti* lines reared in the same
185 insectary were different [10]. To confirm whether these differences were conserved across
186 subsequent generations and environments, we characterized the microbiome of these lines in
187 the insectary that they have been continuously reared in (UTMB) and an insectary at a different
188 institution (LSTM) after being transferred and reared for multiple generations. To compare the
189 microbiome diversity and composition between lines we undertook amplicon sequencing on the
190 V4-V5 region of the bacterial 16s rRNA gene from 20 individuals from each line. Out of the 160
191 individuals sequenced, a total of 79 OTUs were found which represented 44 bacterial genera.
192 Rarefaction curves (Supplemental Figure 1) show that sufficient sequencing depth was
193 achieved.

194

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195 To determine if the microbiome of each *Ae. aegypti* line differed in diversity of bacterial species
196 present, the within line diversity was determined by calculating the Chao Diversity index (Figure
197 1A). The richness of the microbiome was greater in mosquitoes reared in the LSTM insectary
198 compared to those reared in the UTMB insectary (p-value < 0.001). To determine if the
199 community structure of any one line was more closely related to the same line reared at a
200 different insectary, or different lines reared within the same insectary, principal component
201 analysis (PCA) was performed on a Bray-Curtis dissimilarity matrix. Overall, individuals from
202 each line shared a more similar bacterial community structure with other lines from the same
203 insectary (Figure 1B), indicating that the environment and non line-specific factors plays a more
204 influential role in shaping the community structure of the microbiome.
205
206 **Within Insectary Differences**
207 To determine whether the lines differed from each other within the same insectary, alpha and
208 beta diversity was compared between the lines from each insectary. Differences in species
209 richness were observed between the lines at both the LSTM insectary (Figure 2A) (p-value <
210 0.001) and the UTMB insectary (Figure 2C) (p-value < 0.001). To gauge if the lines differed in
211 the bacterial community structure, PCA was performed on a Bray-Curtis dissimilarity matrix. The
212 PCA plots show that the bacterial communities differ between the lines at both the LSTM
213 insectary (Figure 2B) (p-value: 0.001) and the UTMB insectary (Figure 2D) (p-value: 0.001).
214 Strikingly, these results indicate that differences in the bacterial community structure of these
215 four lines are maintained across generations and insectaries.
216
217 To measure the extent of overlap of bacteria identified between the two insectaries, the
218 presence of each genera was compared between each line from each insectary (Figure 3). Out
219 of the 44 identified genera, 23 were present in both insectaries. Six genera are specific to the
220 UTMB insectary, and three genera were specific to the LSTM insectary (Supplemental Table 3).

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221 We examined the relative abundance of the 20 most abundant genera for each line to determine
222 if the bacterial communities were dominated by the same bacterial genera in each line at both
223 insectaries. Different bacteria genera dominated mosquitoes reared in the LSTM insectary
224 compared to the UTMB insectary (Figure 4). However, within both insectaries, the Juchitan lines
225 showed the greatest difference in the relative abundance of different taxa compared to the other
226 lines. All four lines reared in the LSTM insectary were dominated by an ambiguous taxa,
227 *Perfucidibaca*, and *Chryseobacterium*. The four lines reared in the UTMB insectary had a high
228 proportion of *Acinetobacter*, *Psuedomonas,* and *Asaia*.
229
230 We previously reported a correlation between Enterobacteriaceae bacteria and *Serratia* in *Ae.*
231 *aegypti* [10]. Specifically, if the midgut of *Ae. aegypti* was colonized with Enterobacteriaceae,
232 *Serratia* was excluded from colonizing. The robustness of this phenotype after multiple
233 generations and in different environments was measured. We found that this negative
234 correlation between the presence of Enterobacteriaceae and the presence of *Serratia* was still
235 observable in these lines (Supplemental Figure 2).
236

237 **Environment versus Line Specific Differences**

238 To understand if line specific differences are conserved across environments, pair-wise
239 differences in the abundance of each bacterial genera were calculated between lines within and
240 across insectaries (Supplemental Tables 1 and 2). Out of the 44 genera identified, a range of
241 16-38 genera were found in different abundances between lines within each insectary resulting
242 in 36-86% similarity between the lines (Figure 5). To assess whether line specific factors or the
243 environment play a larger role in shaping the microbiome, the percentage of genera differentially
244 abundant was compared for each pairwise comparison within and between insectaries. Lines
245 were more similar to lines from the same insectary than to the matching line in the other

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246 insectary (Figure 5), indicating the environment is more important than line specific factors in
247 shaping the microbiome.
248
249 To assess if lines differ from each other in the same way across environments, pairwise
250 comparisons of lines were performed within each insectary, and genera that showed statistically
251 differential abundances were identified per comparison. If we identify conservation of
252 differentially abundant genera between the same two lines reared in different environments, this
253 would suggest an interaction between specific bacterial genera and the hosts' background
254 across environments. Overall, a range of 11-19 genera were identified as having conserved
255 differences in their abundance between the same lines in each insectary (Figure 6). The
256 proportion of differentially abundant genera between lines that were shared between institutes is
257 dependent on the specific pairwise comparison (Figure 6, Supplemental table 4). In pairwise
258 comparisons between the Galveston vs Iquitos and Galveston vs Juchitan lines, roughly half of
259 the differentially abundant bacteria are conserved across insectaries. In the other four pairwise
260 comparisons, more than half of the differentially abundant bacteria are conserved across
261 insectaries, indicating that the structuring of the microbiome between lines from the same
262 environment is partially conserved across environments.
263
264 **Discussion**
265
266 We explored the contribution of mosquito line and environment in shaping the microbiome
267 composition of *Ae. aegypti*. Having previously observed differences in the microbiome between
268 four lines of *Ae. aegypti* reared under identical conditions in the same insectary [10], we sought
269 to determine if these lines continue to have distinct microbiomes since our previous work, and if
270 this was consistent after rearing in different insectary environments. In accordance with our
271 previous study [10], we observed differences in alpha and beta diversity between the four lines

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272 and interestingly, the microbiome of these lines were still distinct after rearing in separate
273 insectaries. Notably, we found that although the microbiomes of the lines differed from each
274 other within an insectary, any one line was more similar to other lines reared within the same
275 insectary as compared to the same line at the other insectary. Finally, we observed a
276 percentage of differentially abundant genera between lines were shared between insectaries.
277 Together these data demonstrate that while the environment influences the microbiome
278 available to the organisms, host factors still play a role in shaping the microbiome.
279
280 The role of host genotype in determining the microbiome composition has been established in
281 other systems [3-9]. Although the influence of mosquito genotype in shaping the microbiome in
282 the same environment is not well understood, multiple mosquito genes have been identified that
283 influence the composition of the microbiome and gut homeostasis [11, 30-33]. Genes involved
284 in bloodmeal digestion and immune factors can regulate the abundance of the microbiome as a
285 whole [33], or the abundance of specific taxa [30]. Furthermore, the mosquito microbiome can
286 stimulate expression of specific genes to shape the immune status of the mosquito allowing for
287 efficient colonization of specific microbes [32]. In addition to immune genes regulating
288 microbiome composition, metabolic signaling and nutrition status of the mosquito can influence
289 the microbiome [11, 15]. Immunity and nutritional processes are under genetic control and
290 different genotypes of *Ae. aegypti* could result in differences in levels of micro- and
291 macronutrients, which in turn could influence the ability of specific bacteria to colonize that
292 mosquito line. Additionally, genetic variation in immune genes could influence the success of
293 different bacteria in colonizing the mosquito.
294
295 If mosquito line played a more profound role than the environment in determining which genera
296 colonize the mosquito, we would expect the same line at the two insectaries to share more
297 similarities to each other than the other lines within the same insectary, assuming similar taxa

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298 were available to the mosquito across insectaries. Additionally, this would be apparent if
299 pairwise differences in taxa abundance between any two lines within one insectary were
300 conserved in another insectary. We observed the opposite of this where lines within one
301 insectary had more similar abundances of specific genera to each other, compared to the same
302 line reared at a different insectary. We also observed that pairwise differences in the abundance
303 of different genera between lines within one insectary were not shared in another insectary. This
304 lack of shared differences in the abundance of specific genera within a line across institutes
305 suggest that the environment, or the microbes the mosquito has access to, plays a larger role
306 than host specific factors in determining the microbiome. Given that only 25 of the 44 genera
307 identified were shared across insectaries, it is not surprising that line specific abundances of
308 specific taxa are lost across insectaries.
309
310 This study and previous studies observed differences in the microbiome between the lines when
311 reared under identical conditions in the same insectary [10]. This is contrast to our previous
312 work in which midguts from diverse genotypes of *Ae. aegypti* harbored the same microbiome
313 when reared under identical conditions [12]. The discrepancy between these studies could be a
314 result of what tissues were used for microbiome characterization. Kozlova et al. [10] and this
315 study sequenced the microbiome from the whole mosquito, while Dickson et al. [12] sequenced
316 the midgut microbiome. Additionally, it is possible that the environmental bacteria present in the
317 insectary in Dickson et al. [12] could colonize the midguts of all the lines effectively, while this
318 was not the case in the UTMB and LSTM insectaries. Microbe-microbe interactions are
319 important for determining the microbiome composition [17] and perhaps some of the
320 environmental bacteria present in both the UTMB and LSTM insectaries are incapable of
321 colonizing *Ae. aegypti*.
322

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323 Given that the lines of *Ae. aegypti* were not sequenced in this study we cannot conclude these
324 lines represent different genotypes of *Ae. aegypti*. The colonies used in the study represent a
325 similar genetic background in relation to global populations of *Ae. aegypti* [34, 35]. Without
326 sequencing the different lines to confirm genetic differences, we cannot unequivocally determine
327 if the differences we see in the microbiome composition are correlated with mosquito genotype.
328 Although we expect them to be similar genetically, it is likely that there is genetic divergence
329 between the lines and it is reasonable to conclude that the differences between the microbiome
330 between the lines is at least partially genetically controlled.
331
332 The results from this study provide a unique opportunity to tease apart host control of the
333 microbiome in *Ae. aegypti*. By exploiting our new cryopreservation method [36] and swapping
334 the microbiome using our recently developed transplantation approach [37] from both lines and
335 insectaries, the role of the host in controlling the microbiome could be further investigated and
336 provide insight into host genetic mechanisms that underly microbiome composition.
337
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343
344

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345 **Figure Legends.**

346

347 **Figure 1: Diversity of the microbiome in individuals reared at the LSTM or UTMB**
348 **insectaries.** Structure of bacterial communities was determined by deep sequencing the V3-V4
349 region of the bacterial 16*S* gene in adults from 4 different lines of *Ae aegypti* (Galveston,
350 Thailand, Iquitos, Juchitan) reared in two different insectaries at UTMB and LSTM. The Bacterial
351 community structure is represented (A) by the species richness index Chao1 and (B) by
352 principal component analysis of Bray-Curtis dissimilarity index. Mosquito lines reared at LSTM
353 are shown in purple, mosquitoes reared to UTMB are shown in turquoise.

354

355 **Figure 2: Alpha and beta diversity metrics for the *Ae. aegypti* lines reared at the LSTM**
356 **and UTMB insectaries.** (A, C): The species richness (Chao1 index) was calculated from 20
357 individuals from each line (Galveston, Thailand, Iquitos, Juchitan) at each insectary. The level of
358 species richness differed between individuals from the LSTM (p-value < 0.001) and UTMB (p-
359 value < 0.001). (B,D):The dissimilarities between the 4 different lines of *Ae. aegypti* was
360 analyzed by principal component analysis of Bray-Curtis dissimilarity index. The bacterial
361 community structure of the lines differed in individuals from LSTM (p-value = 0.001) and UTMB
362 (p-value = 0.001).

363

364 **Figure 3: Upset plot showing the number of genera that are shared between lines in the**
365 **LSTM and UTMB insectaries.** Each mosquito strain in the different insectary (one per row at
366 the bottom half of the image) is treated as a 'set' with an identified number of bacterial taxa ('Set
367 Size'). The various permutations of intersections are denoted by the ball-and-stick diagram at
368 the bottom of the image, and size of these intersections denoted by the bar graph at the top of
369 the image ('Intersection Size'). Rows are colored based on mosquito strain (middle-left), and
370 further divided into the rearing Insectary (bottom left). 23 taxa are shared across all the different

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371 strains between the different insectaries, constituting a potential 'core' set of bacteria. This is
372 then followed by six (Persicitalea, Janthinobacterium, Rahnella, Uncultured, Luteolibacter,
373 Verrucomicrobium) and three taxa (Sphingopyxis, Burkholderia-Caballeronia-Paraburkholderia,
374 Methyloversatilis) that appeared unique to the rearing insectary. All other permutations of
375 intersects contain two or fewer bacterial taxa.
376
377 **Figure 4: Relative abundance of bacteria in each line.** The dominant bacterial genera are
378 different between insectaries and between lines. The relative abundance of the 20 most
379 abundant genera in shown for 20 individuals from each line at LSTM and UTMB insectaries.
380 Bacterial genera were assigned to OTUs clustered with a 97% sequence identity cutoff and
381 taxonomically classified with the SILVA database.
382
383 **Figure 5: Pairwise comparisons of mosquito lines reared in each insectary.** The
384 microbiomes of mosquito lines reared in the same insectary are more similar compared to those
385 reared in a different insectary. Results from a pairwise differential abundance analysis are
386 shown for each pair of lines as the percent of genera that are significantly different between the
387 pairs after correcting for multiple comparisons. A light blue color indicates a higher degree of
388 dissimilarity between the lines.
389
390 **Figure 6: Few genera have conserved differences in abundance between lines at both**
391 **insectaries.** Venn diagrams show the overlap in specific genera that are differentially abundant
392 between each pair of lines (Galveston, Iquitos, Thailand, or Juchitan) at each insectary (LSTM
393 or UTMB). The ID of genera differentially abundant between each pairwise comparison was
394 compared between insectaries. The shared genera represent a genus that is differentially

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395 abundant between the lines in both environments. The ID of shared genera can be found in
396 supplemental table 4.
397
398
399 **Supplemental figures**
400
401 **Supplemental Figure 1:** Rarefaction curves showing the sequencing depth of each library. The
402 number of species is shown on the Y axis, and the number of sequencing reads is shown on the
403 X axis.
404
405 **Supplemental Figure 2:** Heatmap showing the correlation between bacterial taxa co-
406 occurrence between the different mosquito strains and rearing insectaries. Saturated greens
407 indicate strong co-occurrence between the bacteria taxa, while saturated purple indicates
408 strong exclusion, and whites indicate no positive or negative correlation. We show that
409 *Klebsiella* co-occurs with *Enterobacter* taxa, whilst *Serratia* and *Cedecea* taxa appear to exclude
410 other bacterial taxa.
411
412 **Supplemental Figure 3:** Beta diversity metrics for the *Ae. aegypti* lines reared at the UTMB
413 insectary. The dissimilarities between the 4 different lines of *Ae. aegypti* plus the negative
414 control was analyzed by principal component analysis of Bray-Curtis dissimilarity index.
415
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421 **References**
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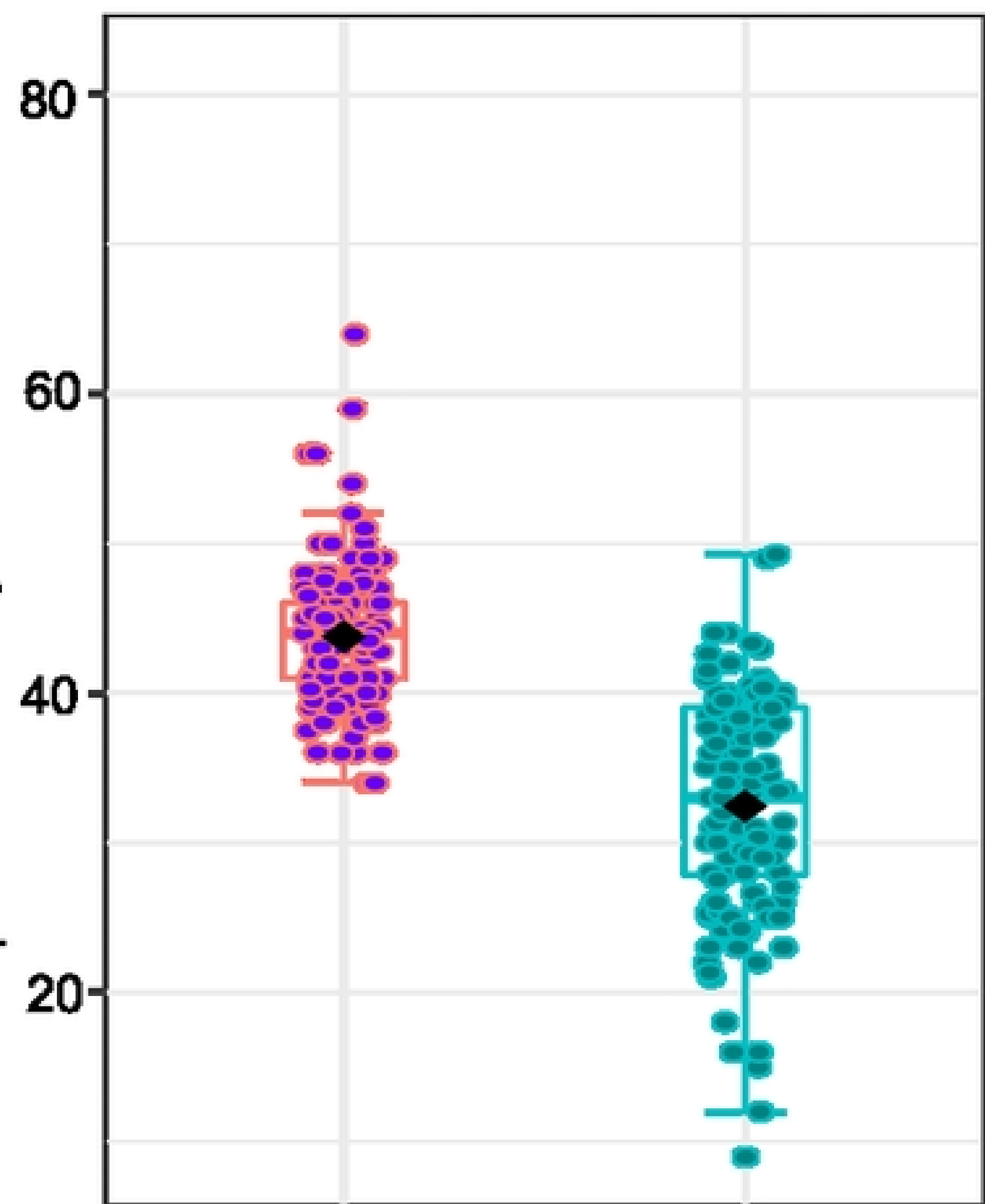
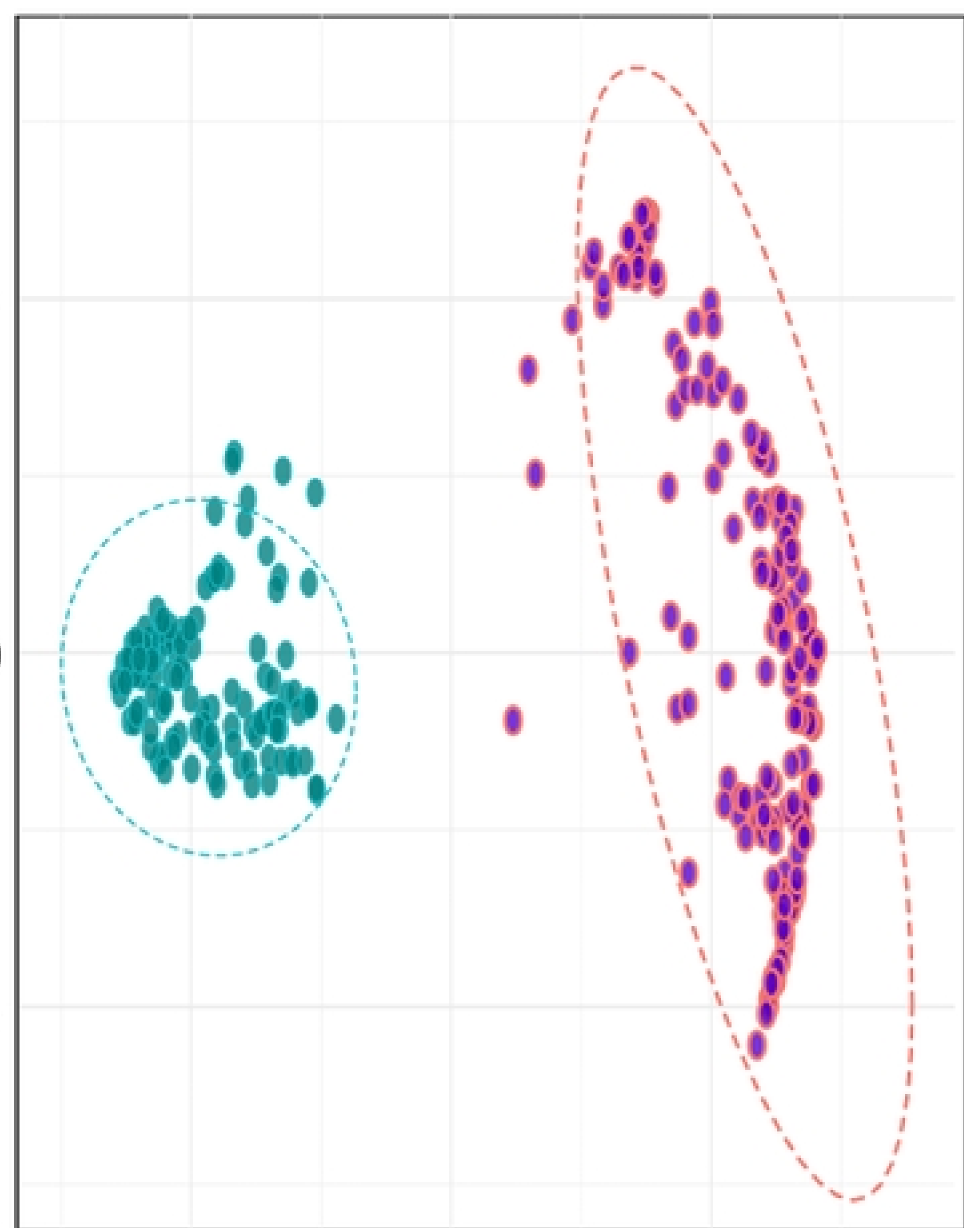
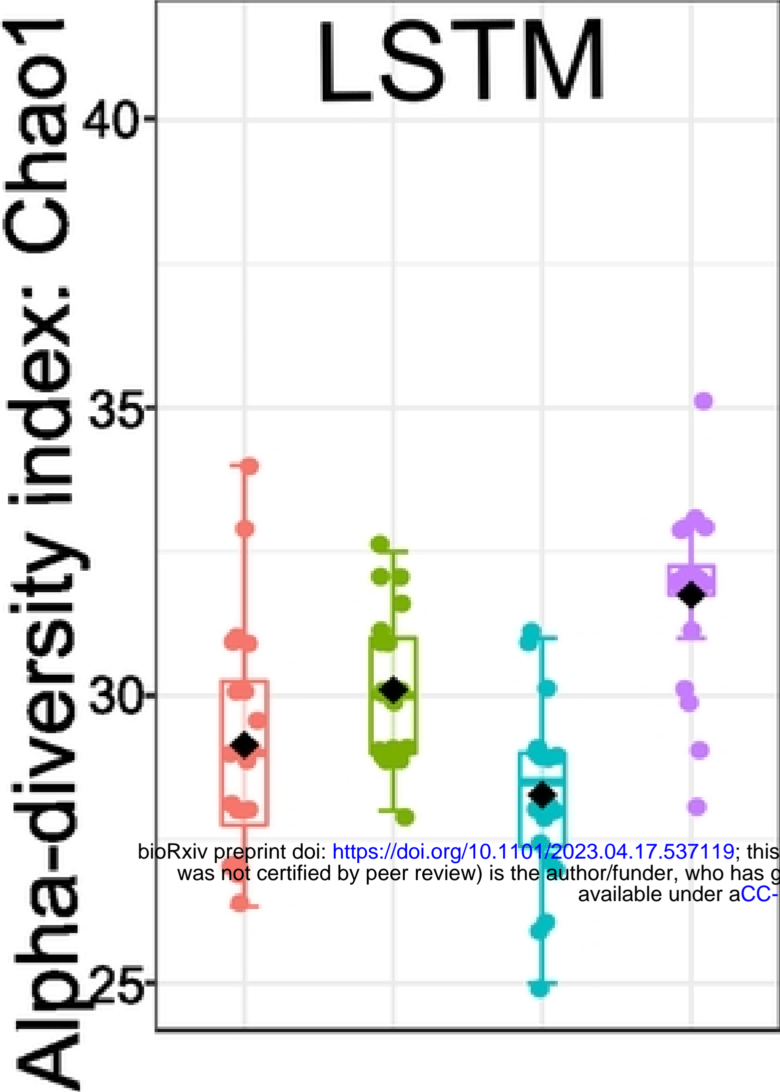
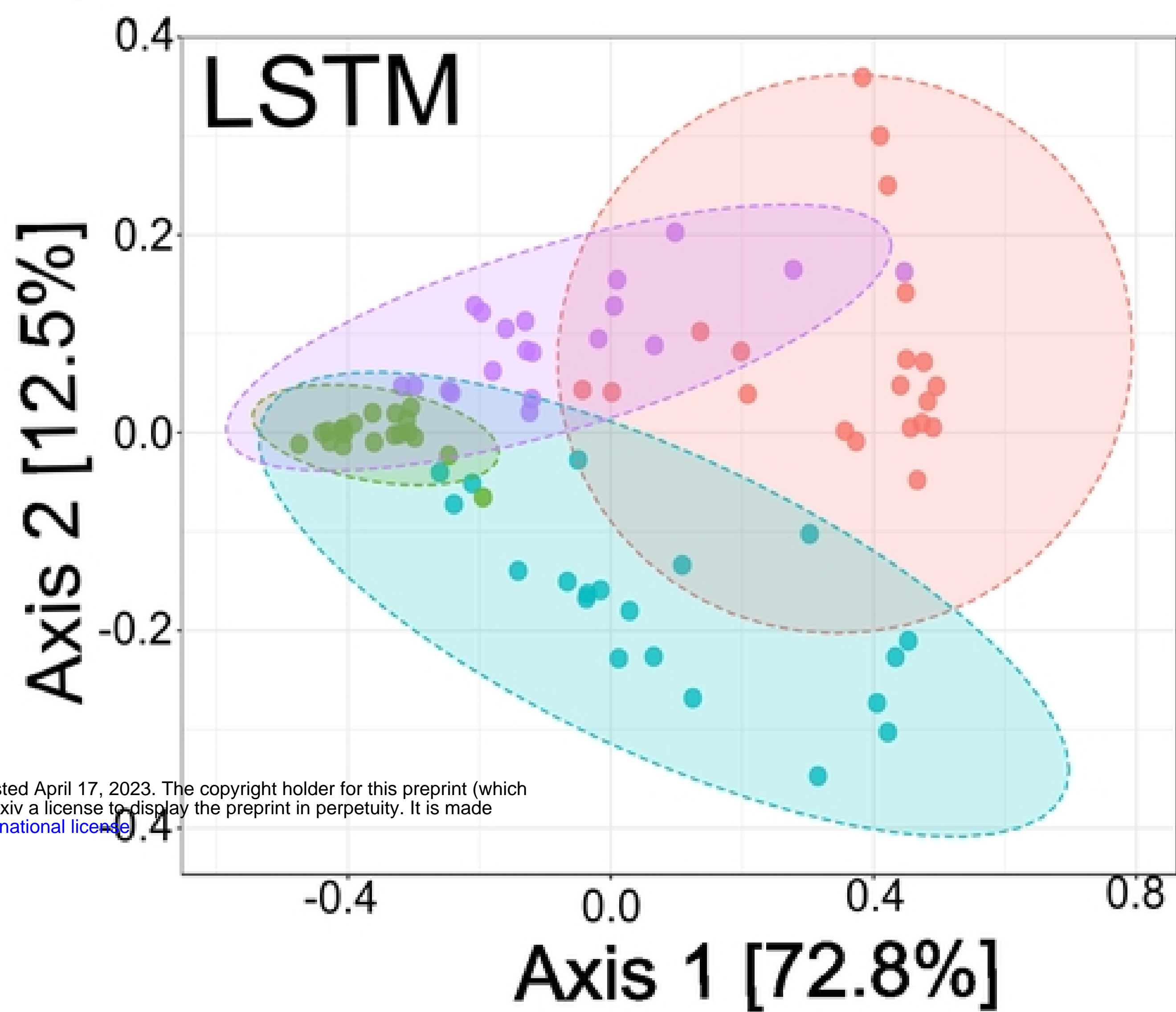




LSTM UTMB

Figure 1





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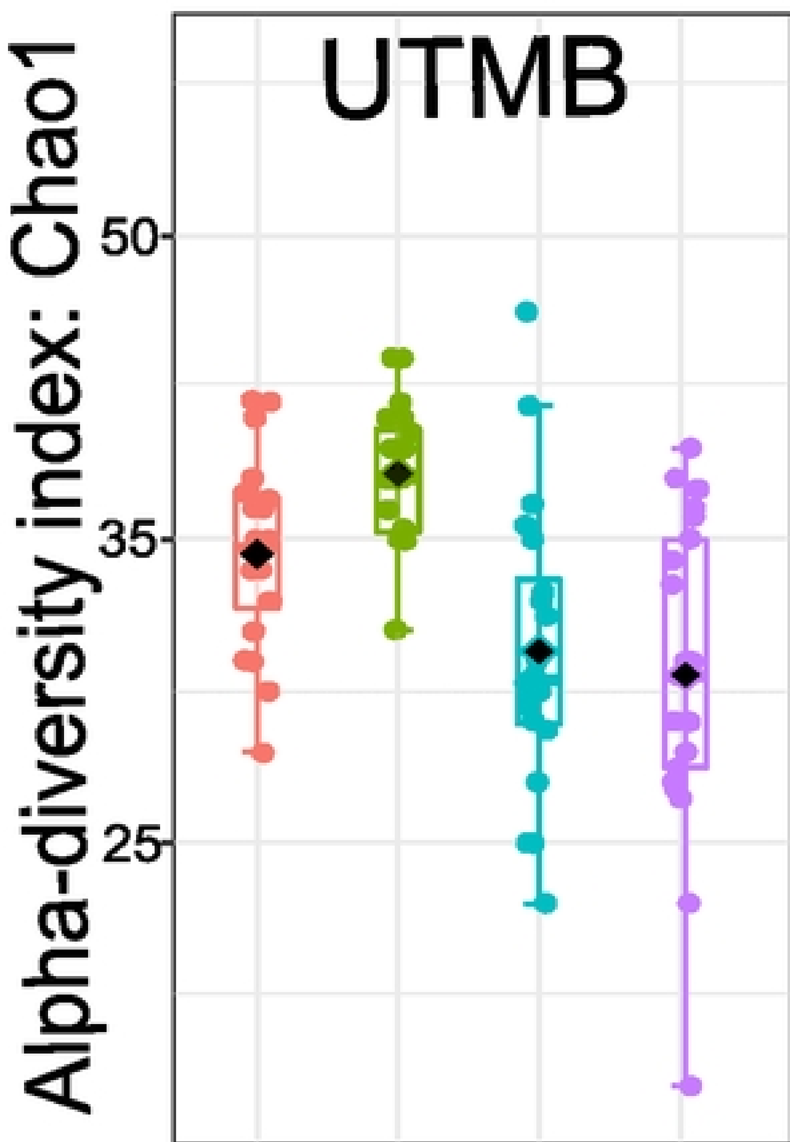

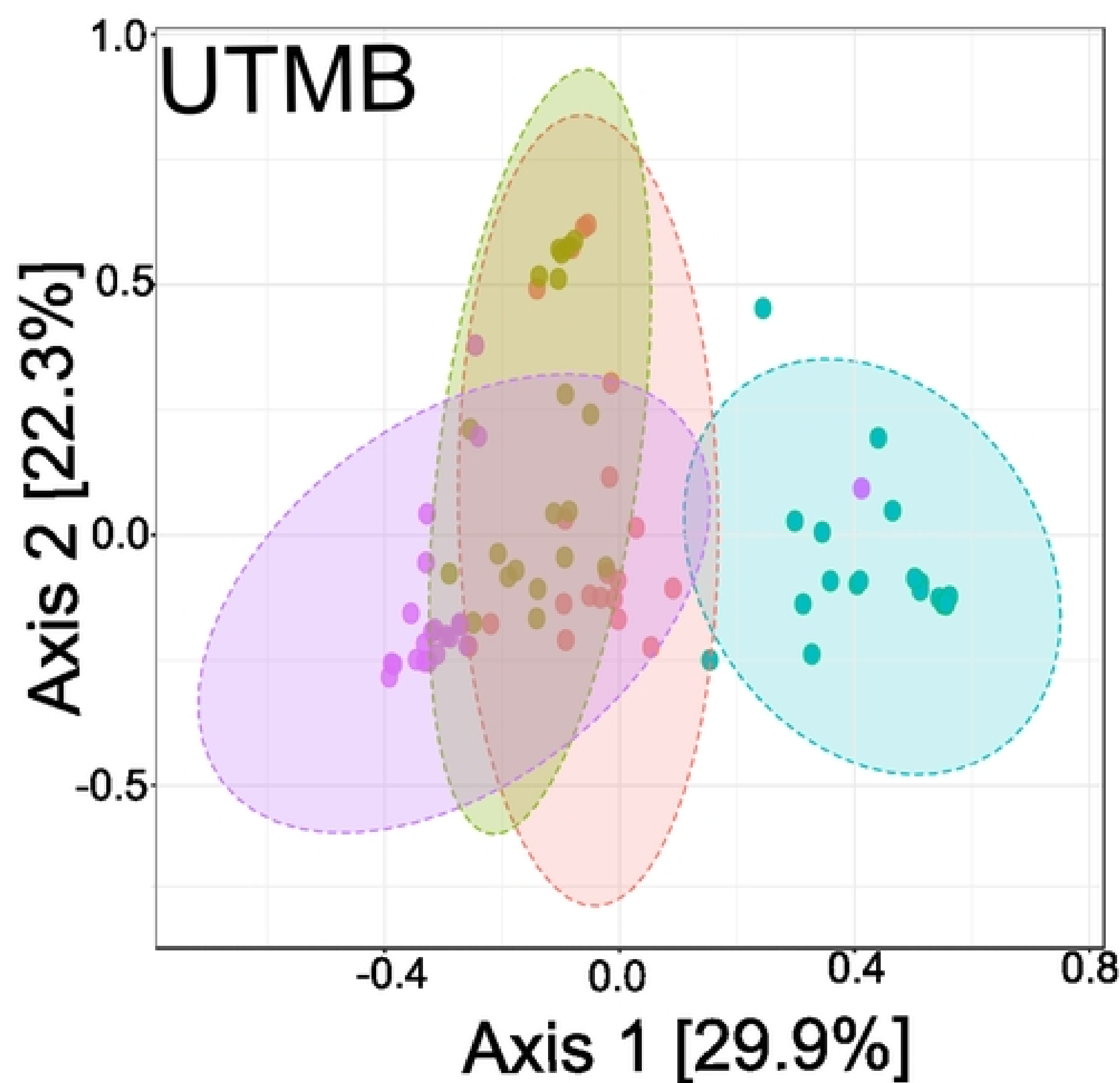

Galveston Iquitos Juchitan Thailand

Figure 2

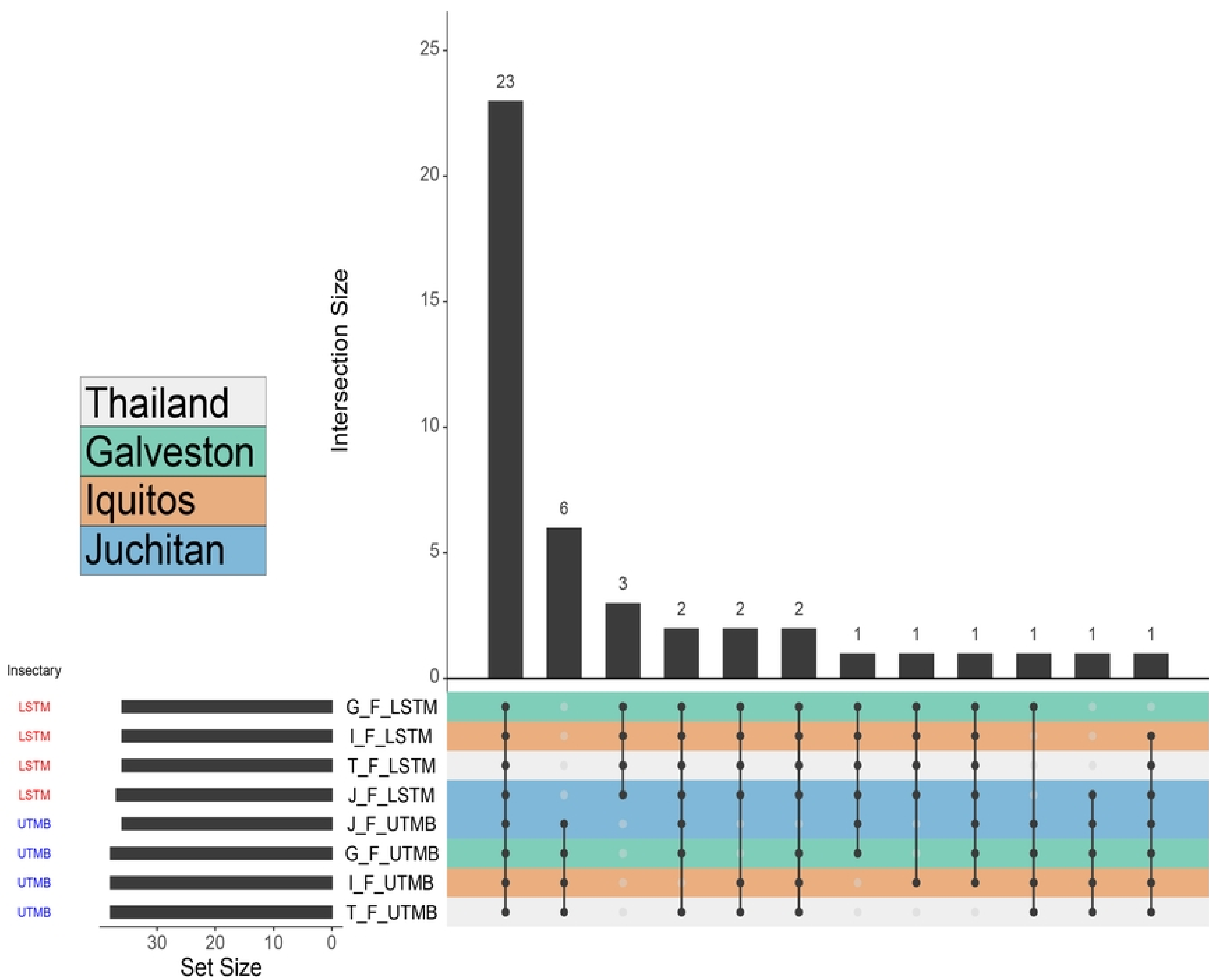

Figure 3

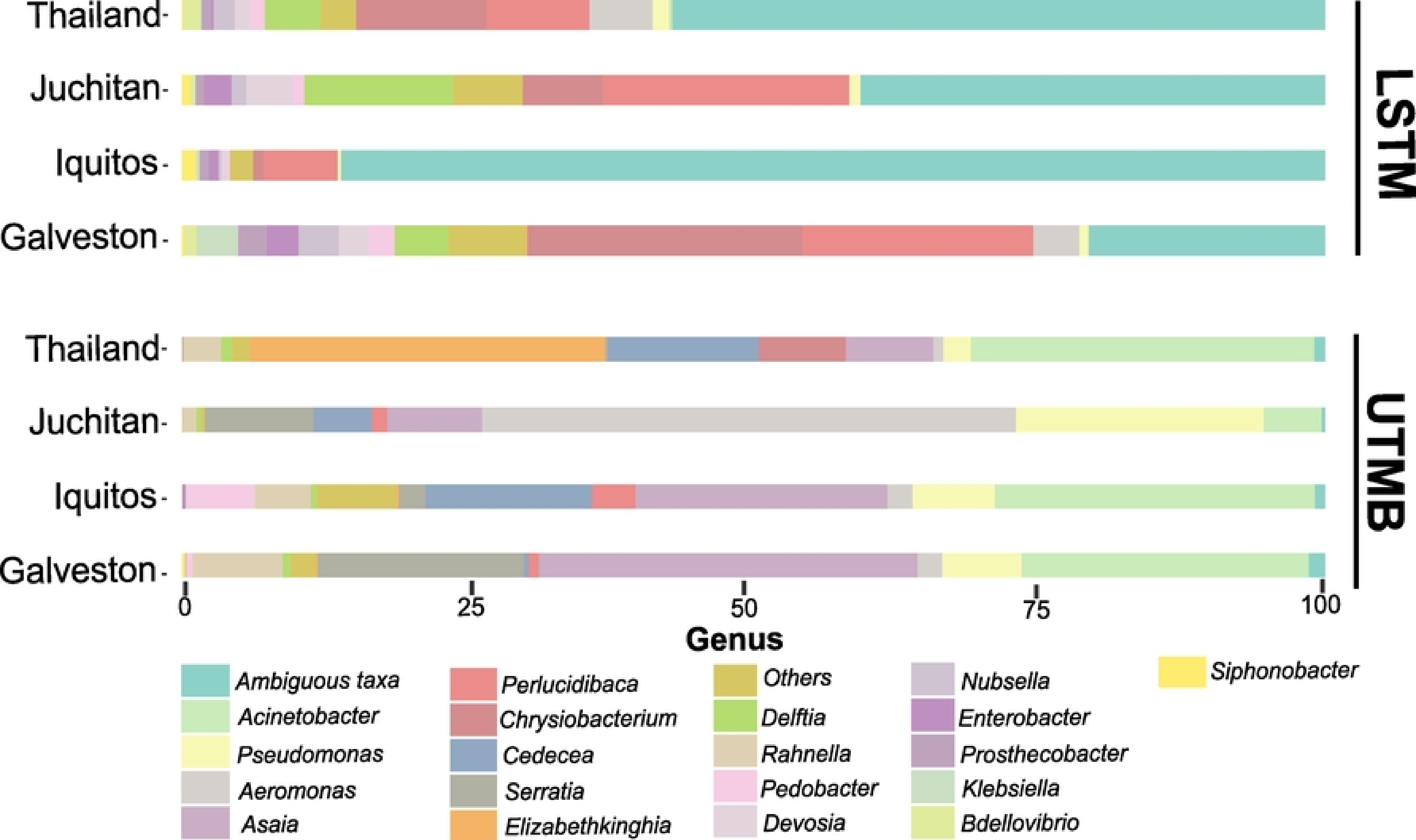

Figure 4

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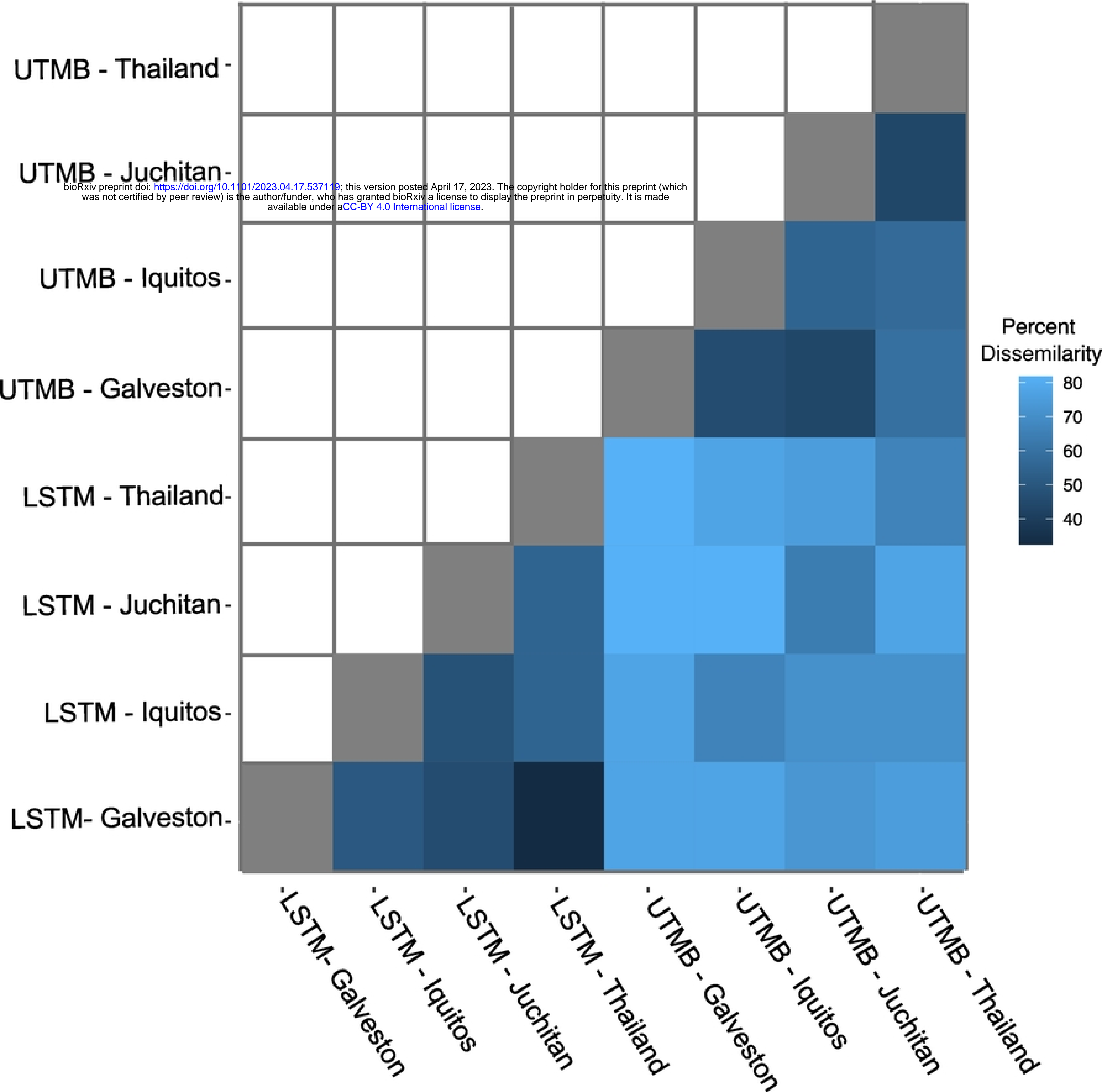
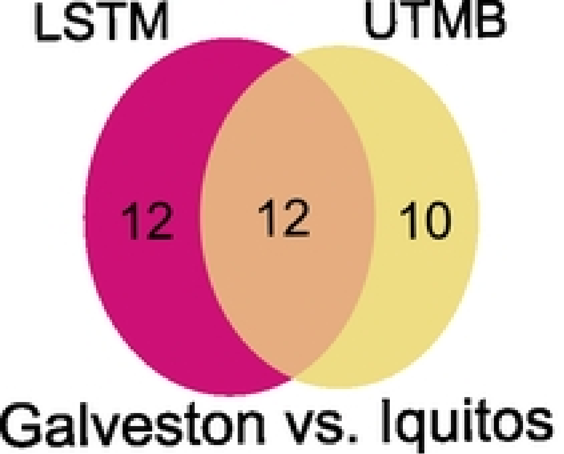
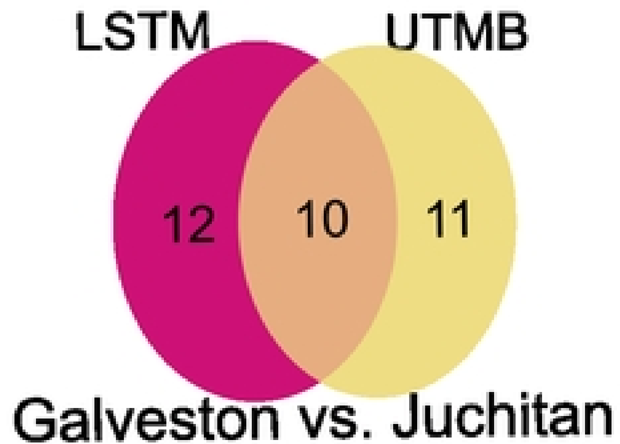
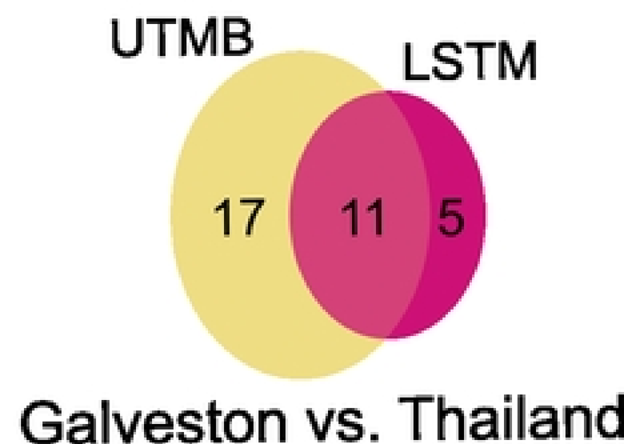
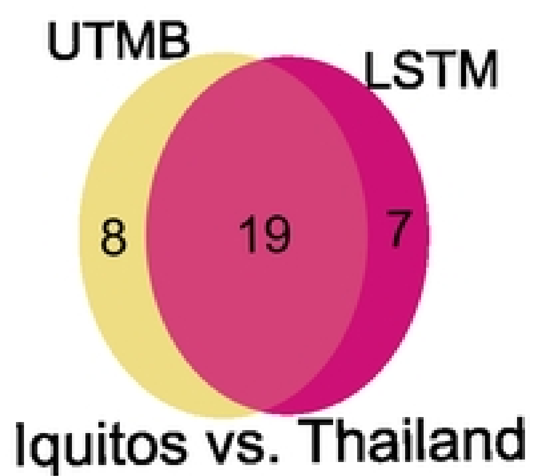


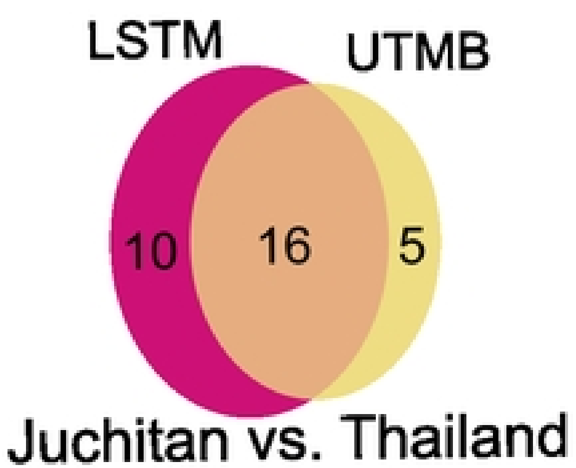
Figure 5











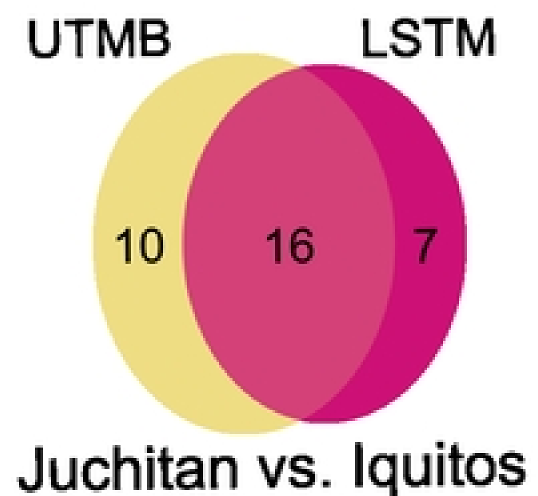


Figure 6