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# Insights on the evolution of vertebrate microbiomes from the analysis of the *Xenopus* frog microbiota across life stages

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## Gut microbial ecology of *Xenopus* tadpoles across life stages

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### Abstract (350 words max)

**Background:** The microorganism world living in amphibians is still largely under-represented and under-studied in the literature. Among anuran amphibians, African clawed frogs of the *Xenopus* genus stand as well-characterized models with an in-depth knowledge of their developmental biological processes including their metamorphosis. We used different approaches including flow cytometry and 16s rDNA gene metabarcoding to analyze the succession of microbial communities and their activities across different body habitats of

27 *Xenopus tropicalis*. We used metagenomic and metatranscriptomic sequencing to evaluate the  
28 metabolic capacity of the premetamorphic tadpole's gut microbiome.

29 **Results:** We analyzed the bacterial components of the *Xenopus* gut microbiota, the adult gut  
30 biogeography, the succession of communities during ontogeny, the impact of the alimention  
31 in shaping the tadpole's gut bacterial communities, the transmission of skin and fecal bacteria  
32 to the eggs. We also identified the most active gut bacteria and their metabolic contribution to  
33 tadpole physiology including carbohydrate breakdown, nitrogen recycling, essential amino-  
34 acids and vitamin biosynthesis.

35 **Conclusions:** We present a comprehensive new microbiome dataset of a laboratory amphibian  
36 model. Our data provide evidences that studies on the *Xenopus* tadpole model can shed light on  
37 the interactions between a vertebrate host and its microbiome. We interpret our findings in light  
38 of bile acids being key molecular components regulating the gut microbiome composition  
39 during amphibian development and metamorphosis. Further studies into the metabolic  
40 interactions between amphibian tadpoles and their microbiota during early development and  
41 metamorphosis should provide useful information on the evolution of host-microbiota  
42 interactions in vertebrates.

43

44 **Keywords** (3-10): *Xenopus*, Amphibian, Microbiome; Metamorphosis, Metagenome;  
45 Metatranscriptome

46

## 47 **Background**

48 Metazoans are vehicles for microbial communities, also named microbiota. The microbiota  
49 and its metazoan host have mutualistic interactions and are thought to adapt and evolve as an  
50 holobiont (Wilson and Sober, 1989; Gill *et al.*, 2006; Zilber-Rosenberg and Rosenberg, 2008;  
51 Bordenstein and Theis, 2015). Several studies have highlighted the importance of the

52 microbiota in the function and development of several organs such as the alimentary canal, the  
53 nervous system and the tegument (Sekirov *et al.*, 2010; Sommer and Bäckhed, 2013; Douglas,  
54 2018). The dynamic interaction between a microbiota and its host is under intense scrutiny  
55 especially for mammalian species and a handful of model organisms (Colston and Jackson,  
56 2016; Douglas, 2019). However, very little is currently known on the biotic and abiotic  
57 interactions between the microbiota of even well-known and classic vertebrate model  
58 organisms such as amphibians (Colston and Jackson, 2016; Douglas, 2019; Rebollar and Harris,  
59 2019).

60 The current paucity of knowledge on amphibian's microbiome is an historical thumb of  
61 nose. Indeed in 1901, Olga Metchnikoff published a note on the influence of microbes on the  
62 development of tadpoles (Metchnikoff, 1901). She concluded that microbes were vital to  
63 tadpoles because they could not complete their development in sterile conditions, and her  
64 results were confirmed by Moro (Moro, 1905). A few years later, these results were challenged  
65 by Eugène and Elisabeth Wollmann, who finally observed that *Rana temporaria* tadpoles could  
66 complete their metamorphosis in sterile conditions (Wollman, 1913). They also reported that  
67 tadpoles could be fed solely with bacteria, pinpointing their nutritive roles (Wollman and  
68 Wollman, 1915). Since that time, only few investigations have addressed the interactions  
69 between bacteria and tadpoles during development.

70 Currently, the best-known amphibian model in biology is the African clawed frog. Several  
71 features of its life cycle can facilitate investigations on the interactions between an animal host  
72 and communities or individual microbial taxa. *Xenopus* have a long lifespan of more than ten  
73 years and several tools are available to study their biology including genomic engineering  
74 (Vouillot *et al.*, 2014). However, there is few data on *Xenopus* microbiota. Mashoof *et al.* used  
75 16S rRNA metabarcoding to describe the bacterial diversity in the gut microbiota of adult

76 *Xenopus* and found that *Firmicutes*, *Bacteroidetes* and *Proteobacteria* were the dominant  
77 bacterial phylum like in other vertebrates (Mashoof *et al.*, 2013; Colombo *et al.*, 2015).

78 Like many amphibians, *Xenopus* life history is characterized by an aquatic external  
79 development. The embryo hatches after one or two days of embryonic development under the  
80 protection of the chorion and of a double-layer jelly coat (Bles, E.J., 1906; Faber and  
81 Nieuwkoop, 1994). The newly hatched embryo is in direct contact with its environment and  
82 usually sticks to the remnants of its jelly coat thanks to secretions from its adhesive gland. The  
83 mouth opens one day later, and after about two more days, the small tadpole starts feeding by  
84 water filtration. Further growth and development continue during four weeks, a period called  
85 premetamorphosis. During the next phase called prometamorphosis, limbs growth is  
86 significant. Finally, the end of morphogenesis is marked by tail resorption during the  
87 metamorphic climax and the adult-shaped froglet is completely formed (Faber and Nieuwkoop,  
88 1994; Brown and Cai, 2007). Most organs, including the gut compartments, are found in both  
89 tadpoles and adults but they experience a complete remodeling that enables the continuation of  
90 their physiological functions in a different ecological niche (Hourdry *et al.*, 1996; Heimeier *et*  
91 *al.*, 2010).

92 Immunologically, post-embryonic development is hallmarked by significant changes  
93 especially regarding mucosal immunity (Robert and Ohta, 2009; Pasquier, 2014; Colombo *et*  
94 *al.*, 2015). In particular, the climax of metamorphosis is hallmarked by high-levels of  
95 glucocorticoids that cause a systemic immuno-depression (Rollins-Smith *et al.*, 1997; Sachs  
96 and Buchholz, 2019). Thus, amphibian metamorphosis is a post-embryonic developmental step  
97 during which host-microbial interactions can play critical roles.

98 Amphibian tadpoles are almost invariably water-dwelling organisms, and most are  
99 microphagous with microbial fermentation contributing up to 20 % of the energy uptake in  
100 bullfrog (Clayton, 2005; Pryor and Bjorndal, 2005; Altig *et al.*, 2007). After metamorphosis,

101 froglets are still microphagous but as they grow they prey on larger animals, with a diet  
102 composed predominantly of chitin-rich insects and other arthropods. The precise timing of  
103 organ and behavioral modifications depends on ecological traits and is known in only a handful  
104 of species, including *Xenopus*. As a *Pipidae*, *Xenopus* tadpoles, metamorphs, froglets and adult  
105 frogs live predominantly in water (Bles, E.J., 1906).

106 Changes in gut bacterial communities during anuran metamorphosis was first studied using  
107 culturing methods and more recently using 16S rDNA gene metabarcoding (Fedewa, 2006;  
108 Kohl *et al.*, 2013; Vences *et al.*, 2016; Chai *et al.*, 2018; Warne *et al.*, 2019; Long *et al.*, 2020;  
109 Zhang *et al.*, 2020). These very interesting studies reported changes in the composition of  
110 microbial communities upon metamorphosis, with differences between species. A single study  
111 expanded its analysis using metagenomic and metatranscriptomic (Zhang *et al.*, 2020). Some  
112 limitations of these studies are that they worked on natural populations of non-model organisms  
113 without genomic resources, they typically sampled a single developmental stage, and they  
114 relied exclusively on DNA extracted from gut samples, yet we are unsure whether this  
115 represents viable bacteria (Emerson *et al.*, 2017).

116 We undertook this study to provide a description of the phylogenetic and metabolic profiles  
117 of the gut microbiota from *Xenopus* tadpoles. We monitored the gut microbiome changes during  
118 development and metamorphosis using 16S rDNA gene metabarcoding, metagenomic and  
119 metatranscriptomic. We investigated the effect of the diet on tadpole's gut bacterial  
120 communities. We identified the communities found in different adult organs, including the skin,  
121 stomach, intestine and rectum. We also investigated the transmission of bacterial communities  
122 between parents and their eggs. Finally, we explored host-gut microbiota metabolic interactions  
123 using functional annotations of the tadpole metagenome.

124

## 125 **Results**

## 126 **Enumeration of bacterial cells in *Xenopus* tadpole's gut during development**

127 We started to assess changes in tadpole's gut communities throughout development by  
128 enumerating bacteria using flow cytometry (Figure 1). We selected six developmental stages  
129 from young tadpoles that just started to feed up to adulthood, through grown-up tadpoles  
130 undergoing metamorphosis (Figure 1A and Figure\_S1). When we analyzed the distribution of  
131 bacterial populations by relative cell size and DNA content, we found that cytometric profiles  
132 differed according to life stages (Figure 1A). We observed distinct bacteria populations in  
133 mature tadpoles compared to young ones (e.g. populations 50-3 and 56-4 absent or extremely  
134 reduced in NF45 tadpoles, Figure 1A and Figure\_S1). We also observed distinct cell  
135 populations in froglet and adult guts (population Juv-4 and Adu-3 for example, Figure 1A and  
136 Figure\_S1). These first observations showed qualitative differences in the gut microbiomes  
137 across life stages.

138 We quantified the total bacterial populations and determined an average of  $1 \times 10^6$  bacteria  
139 per individual at the young tadpole stage,  $2 \times 10^8$  at the prometamorphic stage and  $3 \times 10^7$  in the  
140 froglets (Figure 1B), indicating that the gut bacterial mass increased just after the beginning of  
141 feeding and decreased simultaneously with the gut size reduction occurring during  
142 metamorphosis (Schreiber *et al.*, 2005). We found that the bacterial load changed significantly  
143 during development (ANOVA P-value = 0.041). The increase between pre-feeding and  
144 prometamorphic tadpoles was significant (Tukey adjusted P-value = 0.046). We also observed  
145 a tendency for a decrease between prometamorphic tadpoles and adults (Tukey adjusted P-value  
146 = 0.073); and between prometamorphic tadpoles and froglets (Tukey adjusted P-value = 0.060).

147 We conclude from these cytometric data that there are qualitative and quantitative  
148 differences in the gut microbiota across *Xenopus* life stages. The tadpole's gut is colonized by  
149 a large population of bacteria at the beginning of feeding, and this bacterial population increases

150 during tadpole's development, then reduces upon metamorphosis before increasing again upon  
151 froglet growth.

## 152 ***Xenopus* gut microbial diversity during development and metamorphosis**

153 Since our cytometry results pointed to the presence of an important bacterial population in  
154 the tadpole's guts, we went on to quantify the taxonomic diversity of this microbiota during  
155 development. We set out experiments in which we sampled whole tadpole's guts at different  
156 life stages up to adulthood and quantified bacterial taxonomic diversity using 16S rRNA gene  
157 profiling. We compared 16s rRNA gene profiles amplified from tadpoles at the premetamorphic  
158 stages of development (tadpoles, NF54 to NF56, N=8), at the prometamorphic stages  
159 (prometamorphs, NF58 to NF61, N=6), at metamorphic stages (metamorphs, NF 62, N=5), at  
160 the end of metamorphosis (froglet, NF66, N=6) and from sexually mature adults (N=8). After  
161 filtering low abundance and singleton sequence clusters, we obtained an abundance table for a  
162 set of 666 OTUs. According to rarefaction and species accumulation curves, we obtained a  
163 depth of sequencing sufficient to obtain a fair evaluation of the richness for the most abundant  
164 bacterial species (Figure\_S2).

165 We examined which bacteria constituted these most abundant gut communities (Figure 2  
166 and Figure S3). We observed both global variations in bacterial Phyla composition between  
167 samples from different life stages (Figure 2A) and inter-individual variations between samples  
168 from the same life stage (Figure 2C). In most cases, inter-individual variations were coherent  
169 with the global changes observed with the previous or the next life stage, and for this reason  
170 we pooled the observations from different samples belonging to the same life stage category.

171 At the premetamorphic stage of development, we observed that *Actinobacteria* dominated  
172 the tadpole's gut microbiota (Figure 2A). At this stage, *Proteobacteria* (mostly  
173 *Alphaproteobacteria*) and *Firmicutes* were also abundant while *Bacteroidetes* and  
174 *Verrucomicrobia* contributions were quantitatively modest. The tadpole's gut microbiota



175 composition changed with *Proteobacteria* becoming the major phylum (mostly  
176 *Deltaproteobacteria*) in prometamorphic tadpoles, followed by *Firmicutes*, *Actinobacteria*,  
177 *Bacteroidetes*, *Synergistetes* and *Verrucomicrobia*. The proportion of *Proteobacteria* increased  
178 in metamorphic tadpoles (mostly *Alphaproteobacteria*), *Firmicutes* abundance decreased and  
179 *Bacteroidetes*, *Actinobacteria*, *Synergistetes* and *Verrucomicrobiae* were minor contributors.  
180 Once metamorphosis was completed, the gut microbiome of the froglets changed and  
181 *Firmicutes* became dominant, followed by *Proteobacteria* (mostly *Deltaproteobacteria*),  
182 *Bacteroidetes*, *Actinobacteria*, *Synergistetes* and *Verrucomicrobia*. In comparison, the  
183 composition of the adult gut microbiome was marked by more balanced abundances of  
184 *Proteobacteria* (mostly *Deltaproteobacteria*) and *Firmicutes*, a remarkable abundance of  
185 *Verrucomicrobia* (slightly higher than that of *Bacteroidetes*), and a minor contribution of  
186 *Synergistetes* and *Actinobacteria*. The abundance patterns for *Proteobacteria* were marked by  
187 different contributions of *Alpha* and *Deltaproteobacteria*.

188 These patterns of abundances at the phylum level were attributable to a restricted number  
189 of families (Figure 2B). At the family level, three genera from three families of *Bacteroidetes*  
190 were predominant: *Bacteroides* (*Bacteroidaceae*) with a marked abundance increase at froglet  
191 and adult stage, and dgA-11 (*Rikenellaceae*) with an increase during tadpole growth and then  
192 in adult stages, and *Parabacteroides* (*Porphyromonadaceae*) that increased only at the adult  
193 stage. *Verrucomicrobiaceae* was the dominant family of *Verrucomicrobia* in adult guts, and it  
194 was represented by a single genus, *Akkermansia*. Similarly, a single unknown genus of the  
195 *Synergistaceae* family was found at low abundance in *prometamorphic* tadpoles and in adults.  
196 *Synergistetes* *Microbacteriaceae* was the dominant family of *Actinobacteria* in pre and  
197 prometamorphosis tadpole's guts, and it was represented by a single genus, *Leifsonia*. Seven  
198 families of *Firmicutes* were predominant: *Lachnospiraceae* (several genera) and  
199 *Peptostreptococcaceae* (one unknown genus) with a marked abundance increase at froglet and

200 adult stage; *Planococcaceae* (one unknown genus) that increased only at the adult stage;  
201 *Clostridiaceae 1* (genus "*Clostridium sensu stricto 1*"), *Erysipelotrichaceae* (two genera  
202 *Coprobacillus* and *Erysipelatoclostridium*) and *Ruminococcaceae* (genera  
203 *Hydrogenoanaerobacterium*, *Anaerotruncus*, *Ruminococcaceae*) were found with fluctuating  
204 abundances across the life stages, and finally Family XIII was found only at low abundance.  
205 Eight families of *Proteobacteria* were predominant: *Aeromonadaceae* (genus *Aeromonas*),  
206 *Neisseriaceae* (genus *Vogesella*) and a family of *Rhizobiales* (one unknown genus) dominated  
207 the froglet gut microbiome; *Rhodospirillaceae* (one unknown genus) exhibited a tendency to  
208 decrease across development and were minor in adults; *Desulfovibrionaceae* (genus  
209 *Desulfovibrio*) were a minority at premetamorphosis and were abundant in the other stages,  
210 *Pseudomonadaceae* (genus *Pseudomonas*) and *Bradyrhizobiaceae* (unknown genus) were  
211 found in metamorph's guts, and *Rhodocyclaceae* (genus *Azovibrio*) in prometamorphic  
212 tadpoles.

213 Altogether, we observed changes in the composition of the five most abundant bacterial  
214 phyla making the gut microbiome as development proceeds, both during tadpole growth and  
215 metamorphosis. The major shift during tadpole growth was a reduction of *Actinobacteria* and  
216 an increase of *Proteobacteria*, and the transition to the adult lifestyle was marked by the  
217 increase of *Synergistetes*, *Verrucomicrobia* and *Firmicutes*. This corresponds to a graded  
218 increase of gram-negative bacteria during tadpole growth and development culminating at  
219 metamorphosis before reducing thereafter (Figure 2D).

220 We also analyzed changes of the estimations of OTU richness and phylogenetic diversity during  
221 development (Figure S3, Supplementary\_table\_2). We identified two important and significant  
222 changes: the first was an increase of bacterial species number and diversity that occurred during  
223 tadpole growth between pre and prometamorphosis ( $p=0.000$  using betta and the breakaway  
224 estimation of richness); and the second was the opposite, namely a significant drop in species

225 richness and diversity that occurred at the climax of metamorphosis (Figure S3 and  
226 Supplementary\_table\_2, ( $p=0.000$  using betta and the breakaway estimation of richness). When  
227 focusing on a tadpole-adult comparison, we found that the adult *Xenopus* gut microbiome  
228 contained about twice more OTUs than the tadpole one, and its phylogenetic diversity was 1.8  
229 times higher. These differences in microbiome diversity between the premetamorphic tadpole's  
230 guts and the adult ones were significant using various alpha diversity metrics (Figure S3 and  
231 Supplementary\_table\_2).

232 In parallel to these changes of communities across development, numerous OTU's were  
233 shared between life stages. We found 111 OTUs (17%) present at all stages and representing  
234 22% of the adult gut microbiome and 29 to 38% of the other life stage microbiomes  
235 (Figure\_S4). Another set of 128 OTUs was shared between four life stages, 122 OTU's were  
236 shared between three, 151 between two and only 154 OTUs (23%) were found in only one of  
237 the developmental stages (Figure\_S4). The most prevalent OTUs were detected with a threshold  
238 of very low relative abundances ( $< 5e-05$  %, Figure\_S4B). Nevertheless, we found a set of ten  
239 OTUs that were both common and abundant including an *alpha proteobacterium*, three  
240 *Desulfovibrio*, a *Microbacterioaceae*, a *Clostridium stricto sensu*, two  
241 *Hydrogenoanaerobacteria*, a *Rhizobiales* and a *Lachnospiraceae*. The abundances of the  
242 *Alphaproteobacteria*, one *Desulfovibrio*, the *Clostridium sensu stricto* and the  
243 *Lachnospiraceae* were the most homogeneous.

244 We searched more specifically which OTUs were characterized by a significant change of  
245 abundance across developmental stages using DESeq and identified a set of 22 OTUs  
246 corresponding to five Phyla and 13 known genera (Figure\_S5). The five OTUs most abundant  
247 in premetamorph tadpole's gut but not in adult's gut were a *Microbacteriaceae*  
248 (*Actinobacteria*), a *Rummeliibacillus* and a *Clostridium sensu stricto* 13 (*Firmicutes*), a  
249 *Bacteroides* (*Bacteroidetes*) and an *Alpha-proteobacteria* (*Proteobacteria*). The opposite

250 pattern, i.e. more abundant in adult's guts, was hallmarked by a *Lachnospiraceae* (*Firmicutes*),  
251 a *Synergistaceae* (*Synergistetes*), a *Ruminococcaceae* UCG-014 (*Firmicutes*), a *Rhizobiales*  
252 (*Proteobacteria*) and an *Anaerorhabdus furcosa* group (*Firmicutes*).

253 Significant differences in community composition (beta diversity) were evidenced by  
254 microbial community phylogenetic structure and ordination analysis (Figure S3C,  
255 Supplementary\_table\_2). The mean phylogenetic community structure and its dispersion  
256 differed according to life stages (Figure\_S6). The phylogenetic relatedness of communities  
257 changed significantly during development, with negative and lower mean phylogenetic  
258 diversity values found in metamorph and froglet samples indicating more phylogenetically  
259 clustered microbial communities during metamorphosis. Overall, we conclude that the *Xenopus*  
260 gut bacterial community succession during development and metamorphosis underwent  
261 significant dynamic changes of composition and structure.

### 262 ***Xenopus* microbiota across several gut compartments**

263 To further compare the bacterial diversity along the gut of adult frogs, we dissected the gut  
264 in three parts: stomach, intestine and rectum (N=3 for each tissue sample). In parallel, we  
265 analyzed the bacterial composition of the feces and the skin of these animals using 16S rRNA  
266 gene profiling (N=18 and N=20, respectively). Whereas *Bacteroidetes*, *Firmicutes* and  
267 *Proteobacteria* were the most abundant bacteria in the intestines, the rectum, the feces and the  
268 skin microbiota, we observed that the *Tenericutes* (mostly *Mollicutes*) were the most abundant  
269 in the stomach (Figure 3A, Supplementary\_table\_2). Surprisingly we observed a large quantity  
270 of *Acidobacteria* (28.8%) in only one feces sample. The stomach, intestine and rectum  
271 microbiomes were well differentiated from each other in composition and in structure analysis  
272 (PERMANOVA on bray-curtis dissimilarity F=6.93; R2=0.43; p<0.001, Figure 3B and  
273 Supplementary\_table\_2). Feces and skin microbiomes were characterized by their largest  
274 variability and were well separated along the x-axis in the ordination analysis.

275 Despite these differences, we identified 31 OTUs shared between the three gut sections: 16  
276 were identified as *Bacteroidetes* (including 15 *Bacteroidia*), seven as *Firmicutes* (including six  
277 *Clostridia*), six as Proteobacteria (including five *Desulfovibrio*), one *Parcubacteria* and one  
278 *Synergistetes*. In conclusion, we showed that the bacterial diversity increased along the  
279 anteroposterior axis of the adult *Xenopus* gut, from the stomach to the rectum, and the bacterial  
280 community compositions and structures associated with those gut sections were also  
281 significantly different (Supplementary\_table\_2).

### 282 **Activity of the *Xenopus* tadpole gut microbiome at the onset of feeding**

283 We then asked when bacterial communities started to be active during development and  
284 what bacteria were found at the earliest stages of larval development. When *Xenopus* embryo  
285 hatch, their mouth is not open, the archenteron is well formed and the post-anal gut is open.  
286 Feeding behavior will only start once mouth is formed and gut organogenesis is more advanced,  
287 a few days later (Chalmers and Slack, 1998). We took advantage of this life history trait to  
288 perform a first investigation of the succession of active bacterial communities at early stages of  
289 larval development just after hatching when the mouth is not open (NF 30), before (NF41) and  
290 after feeding (NF48); and in growing premetamorphic tadpoles (NF50 and NF52). We set out  
291 experiments in which we followed-up embryos and tadpoles from two egg clutches up to the  
292 completion of metamorphosis. We targeted active bacteria by collecting RNA from pools of  
293 five whole embryos or tadpoles starting at 24h after fertilization and performing 16S rRNA  
294 gene sequencing.

295 We observed an increase of bacterial species number and diversity up to feeding stage,  
296 followed by a transient reduction at stage 50 (Figure\_S7A). We found 103 OTUs present across  
297 all stages, among which 11 were abundant OTUs (> 1%). At the earliest stage, we observed  
298 that most bacterial activity was attributed to *Proteobacteria* (five OTUs: *Rhodospirillaceae*,  
299 *Aeromonas*, *Rheinheimera*, *Pseudomonas*, *Desulfovibrio*) and *Bacteroidetes* (two *Bacteroides*

300 OTUs, one *Flectobacillus*, one *Rikenella*), that *Firmicutes* (*Clostridium sensu stricto*) and  
301 *Synergistetes* (*Synergistaceae*) were already present while *Fusobacteria* (*Fusobacteriales*) and  
302 *Verrucomicrobia* (*Akkermansia*) were minor contributors (Figure\_S7). We observed a different  
303 dynamic of bacterial community successions between the two clutches. In one experiment,  
304 there was a gradual decrease of *Proteobacteria* between NF30 and NF50, and an increase  
305 thereafter at stage NF52. This was the opposite for *Bacteroidetes*. In the other experiment,  
306 *Proteobacteriae* and *Synergistetes* abundances oscillated with a decrease before and after  
307 feeding while the abundance of *Bacteroidetes* varied in an opposite manner. This fluctuation  
308 was concomitant with a highest abundance of *Synergistetes* that increased after feeding. In both  
309 clutches, *Firmicutes* proportion increased up to feeding stage and decreased afterward.  
310 Altogether our results highlighted the presence of a rapidly developing microbiota along the  
311 first stages of tadpole development, characterized by a shift of the *Proteobacteria/Bacteroidetes*  
312 ratio.

313 We used the same strategy to monitor active bacterial communities in tadpoles at different  
314 stages of metamorphosis using 16S rRNA sequencing on whole digestive tract RNA extracts  
315 (N=2 using pools of five tadpoles). We analyzed the prevalence and the abundance of bacteria  
316 as development proceeded. We found a common set of 33 OTUs present in at least 50 % of the  
317 samples with a relative abundance of 0.001% or more (Figure 4). All these OTUs were also  
318 found at early stages before feeding, and in the gut of tadpoles up to the completion of  
319 metamorphosis (Figure 4B). The five commonest OTUs were also the most abundant (> 0.01%  
320 abundance), and included unknown or ill-defined species: a *Rikenella*, an *Alphaproteobacteria*,  
321 a *Bacteroides*, a *Desulfovibrio* and a *Synergistaceae*. We found a few prevalent bacteria  
322 detected with low abundances such as an *Alphaproteobacteria*, two *Ruminococcaceae*, a  
323 *Rikenella* and an *Haliscomenobacter*.

324 In a complementary analysis, we used a Bayesian algorithm for source tracking to estimate  
325 the proportion of OTUs populating the adult gut that originated from previous stages of  
326 development (Figure\_S7C) (Shenhav *et al.*, 2019). In both experiments, the source of most  
327 OTUs was identified as the feeding tadpole's gut (52 and 95%), a minority was identified as  
328 stemming from the embryos (3 and 19%). Altogether, we identified that only few active bacteria  
329 found in the embryos before the feeding-stage will be later populating the adult gut, and that a  
330 majority of tadpole's active bacteria will pass through metamorphosis and be also present in  
331 the adult gut.

### 332 **Diet shapes *Xenopus* tadpole' gut active communities**

333 Since *Xenopus* tadpoles are suspension feeders, we hypothesized that tadpole's diets could  
334 directly contribute to their active gut bacterial communities (Bles, E.J., 1906). We made an  
335 experiment in which we fed tadpoles with a commonly used micro planktonic-based diet (food  
336 1, N=9) or a custom meal (food 2, N=9), and monitored their gut microbiome composition  
337 using 16S rRNA sequencing on RNAs. The micro planktonic food 1 contained a higher level  
338 of fibers (6.4%) than food 2 (2.6%). The custom meal food 2 was sterile, devoid of chitin and  
339 richer in available glucids 23.9%. The food regime did not change significantly tadpole growth  
340 (Kruskal-Wallis chi-squared = 0.99, df = 1, p = 0.32) or development (Kruskal-Wallis chi-  
341 squared = 0.34, df = 1, p = 0.56, Figure\_S8). We found a total of 485 OTUs shared between the  
342 tadpoles fed with one or another diet, and this was making up the majority of the OTUs found  
343 in each condition: 485 out of 581, i.e. 83% for food 1 and 485 out of 531 i.e. 91% for food 2.  
344 We did not observe significant changes of OTU richness ( $\log_2(W)=3.14$ ,  $p=0.13$ ) and  
345 phylodiversity using Faith's index, ( $\log_2(W)=3.33$ ,  $p=0.29$ ). The differences of alpha-diversity  
346 measured using the Shannon or the Simpson indices were significant ( $\log_2(W)=4.34$ ,  $p=0.001$   
347 and  $\log_2(W)=4.30$ ,  $p=0.004$ , respectively) pointing toward a higher specific richness in tadpoles  
348 fed with food 1 (micro planktonic-based diet). This difference was confirmed by comparing

349 community structures: the mean standardized effect size of mean phylogenetic distances in  
350 communities (SES MPD) was significantly different ( $\log_2(W)=0.69$ ,  $p=0.001$ ), as did beta-  
351 diversity spread (global PERMDISP  $F=13.42$ ,  $df=1$ ,  $N_{perm}=999$ ,  $p=0.001$ ). However, the  
352 mean standardized effect size of mean nearest taxon distance (SES MNTD) was not  
353 significantly different ( $\log_2(W)=3.93$ ,  $p=0.377$ ). The gut bacterial community from tadpoles fed  
354 on food 2 (custom meal) was globally more phylogenetically even (Figure\_S8). The custom  
355 meal was associated with more *Synergistetes* at the expense of *Firmicutes* and *Bacteroidetes*  
356 (Figure 5). The taxonomic and phylogenetic composition of gut bacterial communities were  
357 also significantly impacted by the diet (PERMANOVA on bray-curtis dissimilarity  $F=12.64$ ,  
358  $R^2=0.44$ ,  $p=0.001$  and on MNTD dissimilarity  $F=29.13$ ,  $R^2=0.64$ ,  $p=0.001$ ), as evidenced on  
359 an ordination plot (Figure 5). The relative abundances of all five most abundant Phylum differed  
360 according to the diet, the most notable being the *Synergistetes* that accounted for 0.05 to 0.76%  
361 in the planktonic diet condition and for 0.80 to 17.72% in the sterile diet condition (Figure 5).  
362 We found 82 OTUs whose abundance differed significantly according to diet (Figure\_S8). For  
363 example, *Chitinophagaceae* were abundant in the tadpoles fed with the micro planktonic-based  
364 diet, while a *Sphingobacteriales* was most abundant in tadpoles fed with the artificial diet.  
365 Altogether, we observed that *Xenopus* tadpoles gut microbial communities could be shaped by  
366 their diet without a noticeable impact on growth and development.

### 367 ***Xenopus* microbiome transmission**

368 The way bacterial communities are transmitted across individuals and generations is a recurrent  
369 question in the study of animal microbiomes. We set out an experiment to evaluate the  
370 inheritance of bacterial communities between *Xenopus* parents and their clutch of eggs. We  
371 performed eight independent crosses and sampled the skin and the feces of both parents along  
372 with their egg clutch ( $N=16$ ,  $N=13$  and  $N=8$ , respectively). When we looked at bacterial phylum  
373 diversity, we found that feces contained more *Verrucomicrobia* and *Firmicutes*, while both skin



374 and eggs contained more *Proteobacteria* (Figure 6). In one case, we observed a resemblance  
375 between eggs and the feces of one parent. Globally, the bacterial communities of the skin and  
376 of the eggs were significantly more similar to each other than those from feces in terms of  
377 community structure and composition as evidenced using different metrics (Figure 6,  
378 Supplementary\_table\_2). We also analyzed the proportion of the egg bacteria derived from  
379 adult skin and feces using FEAST, a Bayesian algorithm for source tracking (Shenhav *et al.*,  
380 2019). In five out of the eight crosses, more than 70% of the bacteria found in the eggs did not  
381 come from feces or the skin of the parents. In the three other crosses, we observed that male or  
382 female skin made up to 75% of the egg bacteria. In conclusion, we found that the main driver  
383 of egg bacterial communities was the environment; the skin and the feces of parents were only  
384 minor contributors.

### 385 **Whole shotgun metagenomic and metatranscriptomic analysis of *X. tropicalis*** 386 **gut microbiota**

387 In the final part of our study and since few studies have investigated the genomic functions  
388 of the amphibian microbiome, we used a whole shotgun metagenomic and metatranscriptomic  
389 approach to better characterize the *Xenopus* gut metagenome and its activity in prometamorphic  
390 tadpoles (Figure 7). We filtered out reads mapping to the nuclear or the mitochondrial genome  
391 of *X. tropicalis* and obtained a dataset made of 23.6 and 19.8 millions paired reads using the  
392 metagenomic and metatranscriptomic approach, respectively. We used MATAM, phyloFlash,  
393 kraken2 plus bracken and kaiju to perform taxonomic assignation of these reads against  
394 nucleotidic and proteic databases (Menzel *et al.*, 2016; Pericard *et al.*, 2018; Wood *et al.*, 2019;  
395 Gruber-Vodicka *et al.*, 2020). As expected, bacterial reads were predominant, but we identified  
396 also a few microeukaryotes, including various parasites (Figure S9, Figure S10, Figure S11,  
397 Figure S12). We found low levels of reads assigned to Archaea using kraken2/bracken and  
398 kaiju but these results turned out to be false positives upon closer inspection. Indeed, more

399 discriminant sequence comparison tools such as MATAM, phyloFlash or megablast against  
400 SILVA or the NCBI non-redundant nucleotide databases evidenced that these reads were of  
401 bacterial origin. We also found a few thousands bacteriophage viral sequences using  
402 kraken2/bracken or kaiju but we did not investigate them in more details.

403 Metagenomic and metatranscriptomic sequencing gave similar taxonomic profiles for  
404 bacteria in concordance with the 16S rRNA gene metabarcoding approach, while giving a finer  
405 taxonomic resolution (Figure 7A). Using phyloFlash on metagenomic reads, we assembled 16  
406 near full-length SSU sequences with phyloFlash-SPADES; 31 with phyloFlash-emirge and 33  
407 using MATAM (Figure S9, Figure S10). Using metatranscriptomic reads, we assembled 32  
408 near full-length SSU sequences with phyloFlash-spades; 1498 using phyloFlash-emirge and  
409 440 using MATAM (Figure S11, Figure S12). We observed four main dominant phyla:  
410 *Bacteroidetes* (mostly *Bacteroides*), *Proteobacteria* (mostly *Desulfovibrio* and  
411 *Polynucleobacter*), *Firmicutes* (mostly *Ruminococcus*) and *Actinobacteria* (mostly  
412 *Microbacterium*) (Figure 7A).

413 In conclusion, we confirmed that the *X. tropicalis* tadpole's gut microbiota harbors a large  
414 amount and diversity of bacterial cells, mainly constituted of anaerobic bacteria and common  
415 bacteria at the species level with other vertebrate's gut microbiota.

### 416 ***Xenopus tropicalis* gut microbiota genes catalog**

417 To characterize the *Xenopus* microbiome, we compiled a gene catalog from our  
418 metagenome and metatranscriptome data sets. We assembled the metagenomic sequence reads  
419 using different assemblers and compared the resultant assemblies with metaQUAST  
420 (Mikheenko *et al.*, 2016). In addition we mapped back all metagenomic and metatranscriptomic  
421 reads and also our previously found OTU sequences (Supplementary table 3). We finally  
422 selected one assembly made using idba\_ud, based on the rate of properly mapped reads :  
423 93.36% and 77.58% for DNA and RNA reads, respectively and on the number of OTUs

424 identified : 23 OTU sequences matching perfectly and 78 OTUs matching at 97% identity to at  
425 least one contig. This assembly consisted of 72,183 contigs larger than 500 bp, with a total  
426 assembly length of 161.8 Mbp. The contigs N50 size was 6,100 bp with the largest contig being  
427 640,454 bp long (Supplementary table 3). We then used DASTool to identify 22 bins covering  
428 61.5 Mbp, including 14 bins with high completeness (> 90%) and low redundancy (ie  
429 contamination, < 8%) (Figure 7B, Supplementary table 4). We found a single perfect alignment  
430 of seven OTU sequences in seven different bins, and two bins containing two unrelated OTUs  
431 each.

432 We predicted a total of 141,692 CDS using prokka on the tadpole metagenome assembly.  
433 A subset of 87,012 CDS (61.4%) was classified as hypothetical proteins. At least one  
434 metatranscriptomic read could be mapped on 37,926 CDS. For more than 95.0% of these  
435 “expressed” CDS, the relative expression level was between 0 and 100 reads per kilobase per  
436 million (RPKM) while some were highly expressed with a relative expression higher than  
437 10 000 RPKM.

438 In conclusion, we identified 141,692 CDS from the *X. tropicalis* tadpole gut metagenome.  
439 Using metatranscriptomic sequencing, we revealed that about 27% of these protein sequences  
440 are transcribed and therefore involved in a physiological activity.

### 441 **Metabolic profile of the *Xenopus* gut microbiota**

442 To better characterize the metabolic capacity of the *Xenopus* tadpole's gut metagenome, we  
443 used a functional annotation strategy starting from the 141,692 predicted CDS. Out of these,  
444 34,103 were annotated by prokka with a COG identifier and 32,801 by an EC enzyme number.  
445 We used Minpath to identify 106 KEGG and 1,281 Metacyc metabolic pathways associated  
446 with these known proteins (Ye and Doak, 2009 ; Figure 8A). Enzymatic activities linked to  
447 degradation functions represented 48% of all predicted Metacyc pathways and those linked to  
448 biosynthesis represented 45% (Figure 8A). Pathways involved in fermentation represented

449 0.8% and those involved in detoxification 0.5%. The detoxification potential provided by the  
450 gut metagenome included chloroaromatic compounds and terpenoid degradation. A more  
451 detailed view of the pathways predicted from the tadpole microbiome can be seen on the Krona  
452 plots available in Figure\_S13 and Figure\_S14. Next, we compared the metabolic pathways  
453 encoded by the *X. tropicalis* gut metagenome with those encoded by the *X. tropicalis* genome  
454 (Figure\_S15). To do so, we mapped the EC and KEGG identifiers associated to metagenome-  
455 predicted CDS or to annotated *Xenopus* genes on the Interactive Pathway Explorer (Yamada *et*  
456 *al.*, 2011a). We queried the resulting pathway map using terms corresponding to some of the  
457 numerous metabolites known to be derived from microbiome activity in vertebrates (Figure  
458 8B).

459 In herbivorous and omnivorous organisms, gut microbial communities are known to  
460 degrade complex carbohydrate fibers and provide short chain fatty acids. We identified 6823  
461 genes encoding 205 different families of carbohydrate-active enzymes (89 glycoside hydrolase,  
462 50 Glycosyltransferase, 16 Polysaccharide lyase, 16 Carbohydrate esterase and 32  
463 Carbohydrate-Binding Modules as defined in [www.cazy.org](http://www.cazy.org)) and evidence that acetate,  
464 propanoate and butyrate biosynthetic pathways were active in the *Xenopus* tadpole gut  
465 metagenome (Figure\_S16). We also looked for enzymes involved in nitrogen recycling and  
466 found three CDS involved in N-fixation (*nifH*); 19 genes encoding urease that degrade urea  
467 into ammonia and carbon dioxide (*ureABC*) and two genes encoding uricase homologs (*puuD*).  
468 Thus, tadpole's gut microbes are involved in nitrogen waste recycling to synthesize amino acids  
469 (including essential ones) and polypeptides that can act as nutrient sources. Indeed, we found  
470 that the known essential amino-acids histidine, lysine and tryptophan could be produced by  
471 tadpole gut microbes (Figure 8B, Figure\_S16). Another category of molecular exchange  
472 between the microbiota and its vertebrate host involves the provision of vitamins (Magnúsdóttir  
473 *et al.*, 2015; Dearing and Kohl, 2017). We found evidences that various B-vitamins (folate,

474 cobalamin, pantothenate, riboflavin, biotin, thiamin and pyridoxin) and K-vitamin  
475 (menaquinone) could be synthesized by the tadpole gut microbiota (Figure 8B, Figure\_S14).  
476 Similarly, we identified expressed bacterial genes encoding choloylglycine hydrolase involved  
477 in the biosynthesis of secondary bile acids (Figure 8B). In conclusion, we identified a set of  
478 metabolic pathways from the tadpole gut metagenome that are likely to influence tadpole's  
479 physiology through the action of different metabolites.

## 480 **Discussion**

481 We report that a large community of bacteria develops in the tadpole alimentary canal as  
482 soon as feeding starts and peaks before metamorphosis. While the gut microbiome biomass  
483 diminishes significantly at metamorphosis, it remains important. This probably reflects the  
484 feeding behavior of *Xenopus* at metamorphosis: Naitoh and colleagues reported that filter  
485 feeding can be observed in metamorphs up to stage NF59-NF61, and adult-like feeding starts  
486 at stage NF63 (Naitoh *et al.*, 1989). Thus, there is only a short period of time, two to three days,  
487 during which the *Xenopus* metamorph stops feeding. Also, the gut itself starts to shorten at the  
488 metamorphic stage NF62 and will reduce four-fold to its adult-like length by stage NF66  
489 (Schreiber *et al.*, 2005; Heimeier *et al.*, 2010). This means that adult feeding behavior occurs  
490 before the completion of gut remodeling, and that tadpole and adult digestive physiology  
491 overlap at the end of metamorphosis. In an animal-house setting, we deem it likely that *Xenopus*  
492 froglets continue to eat the available plankton in the rearing aquarium as long as a new diet is  
493 not artificially imposed to them, because they rely upon suction feeding as adults (Bles, E.J.,  
494 1906). More generally, the change of eaten food composition is likely to be gradual depending  
495 on the ecology of the amphibian species considered. For example, Fedewa reported insect  
496 material in the guts of *Bufo terrestris* and *Pseudacris crucifer* at Gosner stage 45-46 (NF64-  
497 NF65) i.e. in metamorph before the completion of metamorphosis, and the interval of fasting  
498 was the beginning of metamorphosis (Gosner stages 42-44, NF60-NF63) (Fedewa, 2006).

499 Our metabarcoding data using DNA and RNA 16S rRNA gene sequencing further  
500 demonstrated that the colonization of the tadpole by microorganisms occurred early during  
501 *Xenopus* tadpole development. Moreover, our results proved that *Xenopus* gut bacterial  
502 community composition changes during tadpole development and metamorphosis. At first, i.e.  
503 before and right after feeding, the change in composition of the microbiota is characterized by  
504 an increase of the *Bacteroidetes* / *Proteobacteria* ratio. The overall diversity of the microbiota  
505 seems to be reduced just before feeding, possibly because of the development and the activity  
506 of innate immune responses of the newly hatched larvae (Pasquier, 2014). After an increase of  
507 the microbiota diversity, once feeding started, there is another step of reduction that we interpret  
508 as the result of an adaptation of the environmental bacterial population to the tadpole's gut  
509 environment. In this period, when the tadpole starts feeding and grows rapidly (NF45 to NF56),  
510 bacterial populations increase 1,000-fold but the microbiota composition remains quite the  
511 same. During metamorphosis, we observed an important diminution of the bacterial abundance  
512 and a change of diversity. Indeed metamorphosis is associated with several modifications of  
513 the gut organs and we expected that gut reduction and neuroendocrine changes would impact  
514 the gut microbiota, as shown previously for other species (Fedewa, 2006; Kohl *et al.*, 2013;  
515 Vences *et al.*, 2016; Chai *et al.*, 2018; Warne *et al.*, 2019; Long *et al.*, 2020; Zhang *et al.*, 2020).

516 The bacterial diversity observed in the *X. tropicalis* gut was consistent with the results of  
517 Mashoof *et al.* on the diversity observed in *X. laevis* gut (Mashoof *et al.*, 2013). Indeed, the  
518 same three phyla dominating the gut microbiota were observed in both analyses. The  
519 dominance of *Firmicutes*, *Bacteroidetes* and *Proteobacteria* is also a characteristic of the  
520 vertebrate gut in general (Colston and Jackson, 2016; Youngblut *et al.*, 2019). Moreover the  
521 *Xenopus* gut microbiota diversity increased following the anteroposterior axis of the gut, as  
522 observed in human gut microbiota (Sekirov *et al.*, 2010). We did not detect Archaea using our  
523 metabarcoding methodology, neither did we identify Archaea in our metagenomic and

524 metatranscriptomic data. While we expected to miss Archaea in metabarcoding sequences  
525 because we relied on mainly bacteria-targeting primers, the lack of Archaea sequences in the  
526 shotgun sequences was more surprising. This constitutes a limitation of our survey and an  
527 interesting subject for further investigation in light of the account of methanogenesis in adult  
528 *Xenopus* (Saengkerdsub and Ricke, 2014).

529 We found that the abundance of several *Bacteroides*, *Bilophila* and *Lachnospiraceae*  
530 bacteria increase during tadpole development and metamorphosis. These bacteria are well-  
531 known to be abundant in the gastro-intestinal tracts of numerous vertebrates, including humans,  
532 and characterized by their resistance to high concentrations of bile salts or their bile acid  
533 hydrolase activities (Song *et al.*, 2019). In addition, we identified seven CDS encoding an  
534 enzyme involved in secondary bile acid biosynthesis. We therefore propose an interpretation of  
535 our findings in light of bile acids being key molecular components regulating the gut  
536 microbiome composition during amphibian development and metamorphosis. Bile acids are  
537 small molecules produced mainly in the liver from cholesterol and further metabolized in the  
538 gastro-intestinal tract. They are known to act as molecular messengers between the host and  
539 their gut microbiota: on the host side, they play a role in numerous metabolic pathways via their  
540 cellular receptors and on the microbiome side they can modulate bacterial growth directly and  
541 indirectly (Ridlon *et al.*, 2014; Wahlström *et al.*, 2016). Bile acids can act directly on the gut  
542 microbiome because they have intrinsic antimicrobial activity and indirectly because their host  
543 cellular receptors regulate antimicrobial peptides genes. In turn, some bacteria encode bile  
544 hydrolases that enable the production of secondary bile acids (Joyce *et al.*, 2014; Song *et al.*,  
545 2019). Therefore, we can consider bile acid composition as a trait of strong evolutionary  
546 significance since it links nutrition, gut, liver and gill physiology, host genome evolution and  
547 the gut microbiome composition (Reschly *et al.*, 2008; Hofmann *et al.*, 2010; Ridlon *et al.*,  
548 2014). In amphibians, bile acids are present in tadpoles but they increase in quantity and

549 complexity after metamorphosis (Anderson *et al.*, 1979; Cole and Little, 1983; Reschly *et al.*,  
550 2008). It is known that bile acids composition changes and complexity increases due to changes  
551 in host gene expression. We hypothesize that ontogenetic and endocrine regulation of  
552 amphibian host genes such as the bile acid nuclear receptor (Farnesoid X receptor, FXR;  
553 NR1H4), or its surface G-protein coupled receptor (TGR5) could be initial regulators of gut  
554 microbiome composition. FXR, an intra-cellular bile acid-sensing transcription factor of the  
555 nuclear factor superfamily, regulates bile acid and lipid homeostasis, and other genes such as  
556 FOR in *Xenopus* may play similar roles using different ligands (Seo *et al.*, 2002; Reschly *et al.*,  
557 2008; Frisch and Alstrup, 2018). It is noteworthy that in mice TGR5 has been shown to link  
558 bile acid and thyroid hormone signaling by promoting intracellular thyroid hormone activity  
559 via the induction of deiodinase 2 in brown adipose tissue (Watanabe *et al.*, 2006). In future  
560 studies, manipulation of gene activities, of bile acid composition and concentration and of the  
561 microbiome could be used to unravel the diversity and the evolution of these regulations in  
562 amphibians and other vertebrates.

563 We provide evidence that the diet of *Xenopus* tadpoles affected the diversity of their gut  
564 microbiome and thus that the mucosal immunity of tadpoles can interact with a variety of  
565 bacterial communities (Figure 5). Tadpoles fed a micro planctonic-based diet had a gut  
566 microbiome with a higher specific richness compared with tadpoles fed a sterile flour-diet. The  
567 latter diet was associated with a lack of *Chitinophagaceae* involved in chitin degradation. These  
568 results contribute to the growing body of evidence that diet as an environmental factor can  
569 affect the composition of the gut microbiota.

570 Maternal transmission of microbes in animals is a well-known phenomenon but its  
571 documentation in amphibians is restricted to only few examples (Funkhouser and Bordenstein,  
572 2013; Walke *et al.*, 2014; Warne *et al.*, 2019). In amphibians like in other animals, the  
573 transmission of microbial communities can be horizontal, vertical or environmental. The



574 vertical transmission mode may depend critically on the reproductive behaviour of the animals.  
575 In *Xenopus*, the natural mating behaviour of the couple in amplexus involves looping dances  
576 from the bottom to the surface. Eggs are mostly laid by the female when she is near the surface,  
577 and immediately fertilized by the male. These movements may lead eggs to stick to the skin of  
578 either parent, and since the skin of amphibians harbors a rich microbiota, it can constitute a  
579 vector of vertical transmission from either parent. In addition, the eggs are laid through the  
580 female cloaca, and the sperm is also emitted by the male cloaca. Since the cloaca is a common  
581 anatomical structure shared by the reproductive, excretory and digestive systems, the microbial  
582 communities of these three systems may be transmitted to the eggs. Here we sampled the skin  
583 and feces of both parents to quantify the transmission to the eggs. Quantitatively and using the  
584 Bayesian algorithm of source tracker, we found that the bacteria found on the eggs were mostly  
585 coming from the environment, and the second source was the skin of either parent. It was  
586 notable that a *Chitinimonas* species, a bacterium known to synthesize the antifungal Violacein,  
587 was one of the OTU transmitted from both parents in all crosses. This is relevant in the context  
588 of *Saprolegnia* egg infections and chytridiomycosis. Recently, Warne and collaborators  
589 reported that changes of the woodfrog egg microbiota influenced the bacterial communities at  
590 later larval stages (Warne *et al.*, 2017, 2019).

591 *Bacteroidetes*, *Firmicutes* and *Proteobacteria* were slightly more represented in the  
592 metatranscriptome than in metagenome highlighting that those bacteria were present and active  
593 in the tadpole gut. Actinobacteria were less abundant in metatranscriptome than metagenome,  
594 showing a reduced activity. On the contrary, we found that *Verrucomicrobia* were very active.

595 Our data highlight the important capacity of the microbiota to complement the metabolic  
596 pathways of the *Xenopus* genome. We found that the gut microbiota of tadpoles synthesizes  
597 short chain fatty acids, essential amino acids and vitamins and recycle nitrogen waste products.

598 The need to perform investigations on animals from natural populations has rightly been  
599 underscored, for example by an interesting review of Sarah Hird (Hird, 2017). Yet we face  
600 several methodological difficulties when it comes to sample natural populations. In some  
601 studies, animals have been captured in the wild and raised thereafter in a laboratory setting. The  
602 current global threats on amphibians makes such studies difficult to realize and restricts  
603 severely the breadth of species that can be studied. In addition, the capture of vertebrate animals  
604 induces a variable amount of stress, leading to different responses including an  
605 immunosuppression (Rollins-Smith *et al.*, 1997; Narayan, 2013). While the effect of captivity  
606 on amphibian microbiomes has been documented, there is a ample room for possible  
607 explanations (Becker *et al.*, 2014; Loudon *et al.*, 2014; Bataille *et al.*, 2016). Also, parasitism  
608 is common in natural populations, and it can represent a hidden variable of considerable  
609 importance in microbiome studies. For example, the mutualism between the nematode  
610 *Gyrinicola batrachiensis* and *Rana catesbeiana* leads to a quicker tadpole development and has  
611 been linked to the hindgut fermentation capacity (Pryor and Bjorndal, 2005). Studies on  
612 laboratory animals for which rearing conditions are more uniform, with the potential for a better  
613 control of parameters and for reproducing experiments in different laboratories are definitively  
614 needed to make the link with investigations on natural populations. In this regard, *Xenopus*  
615 represents an interesting model since it is a widespread anuran with more than 20 species  
616 described in sub-Saharan Africa and classified of least-concern according to IUCN, and there  
617 is a vast body of biological knowledge relating to its development, its physiology and its  
618 genetic. We hope that our study will constitute a background knowledge enabling future work  
619 in the study of amphibian-microbial symbioses.

620 There were several important limitations to this study. First, marker gene and metagenomic  
621 studies are replete of confounding aspects and more studies will be needed to reproduce our  
622 observations using alternative technologies and more samples to explore inter-individual

623 variability (Pollock *et al.*, 2018; Bharti and Grimm, 2019; McLaren *et al.*, 2019). This is  
624 especially needed regarding the construction of a metagenome gene catalog. Second, we  
625 analyzed the microbiome of *Xenopus* tadpoles in a laboratory setting and this is likely to differ  
626 from animals sampled *in natura*. Third, we did not survey Archaea nor microeucaryotes that  
627 are important players of the gut microbiome in other vertebrates and this will need further more  
628 dedicated efforts.

629

## 630 **Conclusions**

631 In summary, we described the bacterial components of the *Xenopus* gut microbiota, the  
632 adult gut biogeography, the succession of communities during ontogeny, the impact of the  
633 alimentation in shaping the tadpole's gut bacterial communities, the transmission of skin and  
634 fecal bacteria to the eggs. We also identified the most active gut bacteria and their metabolic  
635 contribution to the tadpole physiology. Keeping in mind the advantages of the *Xenopus* model,  
636 our data provided evidences that *X. tropicalis* raised in animal facilities is highly suitable for  
637 host-microbiota studies. Our study contributes to the growing body of research on the study of  
638 bacterial symbiosis in amphibians and on the evolutionary associations between amphibians  
639 and their microbiota. Further studies on the dynamics of the *X. tropicalis* gut microbiota during  
640 early development and metamorphosis should provide useful information on the evolution of  
641 host-microbiota interactions in vertebrates.

642

## 643 **Methods**

### 644 **Animals and animal husbandry**

645 We used *Xenopus tropicalis* frogs from the TGA and Sierra Leone strains and we let them  
646 reproduce using induced natural mating as described in the supplementary

647 Extended\_materials\_and\_methods. Parents were transferred back to their housing tank while  
648 the embryos were let in the mating tanks. Tadpoles were reared in static water throughout  
649 embryogenesis and metamorphosis. Tadpoles and adults were euthanized with tricaine methane  
650 sulfonate pH 7.5 at 5 g.L<sup>-1</sup>. We used the Nieuwkoop and Faber table of development to identify  
651 tadpole's developmental stages (Faber and Nieuwkoop, 1994).

## 652 **Tissue sampling**

653 We dissected euthanized tadpoles or adults in sterile amphibian phosphate buffered saline  
654 (aPBS) to collect the gastro-intestinal from the stomach to the rectum. For the sake of  
655 simplicity, we used the term “gut” to refer to the whole gastro-intestinal tract of tadpoles or  
656 adults throughout the manuscript. Whole embryos and early tadpoles were abundantly rinsed  
657 using aPBS before further processing. The skin of adult frogs was sampled after having  
658 abundantly rinsed the animals using aPBS. Sampling was performed by swabbing the dorsal  
659 and ventral skin areas using a sterile cotton swab. Feces were collected using a sterile Pasteur  
660 pipette from frogs that were individually housed in aPBS after reproduction. Samples of two-  
661 hundred eggs including their outer and inner jelly coat were collected using a sterile Pasteur  
662 pipette from the reproduction aquarium, and washed three times in sterile aPBS. All tissue  
663 samples used for DNA extraction and metabarcoding were then immersed in at least ten  
664 volumes of ethanol and stored at -20°C until DNA extraction. All tissue samples used for RNA  
665 extraction and metabarcoding were kept on ice and then processed for tissue lysis as explained  
666 in the Supplementary Extended\_materials\_and\_methods.

## 667 **Bacterial purification from intestinal tracts**

668 We homogenized each freshly dissected intestinal tract in 500 µL of ice-cold PBS using a  
669 Potter-Elvehjem homogenizer. We then concentrated the bacteria extract by successive  
670 filtrations on 40, 20 and 5 µm filters (nylon 40 µm cell strainer from BIOLOGIX; 20 µm net  
671 ring from Pharmacia Fine chemicals; Whatman® Puradisc 13 syringe filters 5 µm from

672 SIGMA-ALDRICH®). Next, we harvested the cells by centrifugation at 13 000g for 15 min at  
673 room temperature. These pellets were then used for nucleic acid extraction (details in  
674 Supplementary Extended\_materials\_and\_methods) or fixation and microscopy (for quality  
675 control) or flow cytometry analysis.

## 676 **Flow cytometry analysis**

677 We prepared triplicate samples from tadpoles at stages NF45-48, NF51-54, NF56, NF60-  
678 61, 15 days froglets and adults. Each sample was made from five tadpole's guts per replicate  
679 for the NF45-48 and NF51-54 stages, single individuals were used for later developmental  
680 stages. We fed the tadpoles two days before the first time point (stage NF45-48), and ten days  
681 before sampling the froglets. We prepared the filtered bacterial cell samples as described  
682 previously and we fixed the resuspended bacterial pellet in 1.0% paraformaldehyde at 4°C  
683 during 2 h. We analyzed samples prepared by mixing bacterial samples with fluorescent beads  
684 (Flow-Count™ fluorospheres, 10 μm diameter, 1010 beads/μl) to obtain a final concentration  
685 of 20 beads/μl. Bacterial samples were stained using propidium iodide (PI) with a MoFlo®  
686 Astrio™ cytometer (BECKMAN COULTER). Excitation was made at 488 nm and 561 nm for  
687 scattering (Forward Scatter, FSC; Side Scatter, SSC) and PI respectively. Emission of PI was  
688 collected through a 614/20-nm band pass filter. Each measure was done in triplicate to compute  
689 a mean value and the standard deviation for each biological replicate sample. Plots were  
690 normalized on 200,000 gated events for the analysis of cell populations.

## 691 **Metagenome and metatranscriptome sequencing**

692 We prepared genomic DNA (gDNA) and RNA from freshly prepared 5 μm filtered gut  
693 samples prepared as previously described and obtained from five *X. tropicalis* tadpoles at stage  
694 NF56 raised in the same aquarium. Metagenomic and metatranscriptomic library construction  
695 and sequencing were performed by the Beijing Genomics Institute (BGI) using an Illumina®  
696 HighSeq™ 2500. The insert sizes of the metagenomic and metatranscriptomic libraries were

697 170 bp and 180 bp, respectively. We obtained 47,279,786 and 40 000 000 high quality 100bp  
698 paired end sequences for metagenomic (ERS716504) and metatranscriptomic (ERS716505),  
699 respectively.

## 700 **16S rRNA and 16S rDNA library construction and sequencing**

701 We prepared genomic DNA (gDNA) from dissected tissue samples that were fixed in  
702 ethanol and stored at -20°C before processing. RNA was prepared from fresh tissue. gDNA or  
703 RNA were used as template for 16S Ribosomal RNA (16S rRNA) or DNA (16S rDNA)  
704 amplification. A detailed list of samples used for the 16S rDNA and rRNA library construction  
705 is given in Supplementary\_table\_1. A first set of three replicate PCR reactions was performed  
706 to amplify the V3-V4 variable region of the 16S rDNA and rRNA; the PCR condition details  
707 and oligonucleotide sequences are given in Supplementary Extended\_materials\_and\_methods.  
708 The PCR products were sent to the GeT sequencing facility (France Génomique, INRA,  
709 Toulouse) for library preparation and sequencing. PCR products were purified and quantified  
710 by spectrophotometry and a second PCR was performed to integrate a barcode index. The  
711 barcoded PCR products were pooled and their quality was checked using capillarity  
712 electrophoresis and quantitative PCR. Finally, the PCR products were sequenced using a Miseq  
713 (supplied by Illumina®). We obtained a total of 3,647,548 clean read counts from 132 samples.  
714 The metadata and number of sequences obtained by sample is given in Supplementary\_table\_1.

## 715 **16S rRNA and 16s rDNA sequence analysis**

716 We sequenced 62 samples and obtained an average of 25,218 assembled paired-end  
717 sequences per sample, with a minimum of 8,134 and a maximum of 66,578. We used the FROG  
718 pipeline for OTUs identification and taxonomic affiliation (Escudié *et al.*, 2018). Overlapping  
719 reads were assembled and sequences were dereplicated before clustering using the SWARM  
720 algorithm. Chimeric sequences were then removed and OTUs were filtered on abundance for

721 at least 0.005%. Taxonomic affiliation of the OTUs was performed based on the 16S SILVA  
722 database (V.123) using blast and RDP classifier.

723 We used script implemented in R to perform different microbiome community analysis  
724 (provided on GitHub at <https://npollet.github.io/metatetard/>) (R Core Team, 2019). Source-  
725 sink analysis was performed using Feast (Shenhav *et al.*, 2019).

## 726 **Taxonomic assignment for metagenome and metatranscriptome sequences**

727 We used MATAM and phyloFlash to extract and assemble full-length or near full-length  
728 16S and 18S rRNA gene sequences from the same set of filtered reads as the one used for the  
729 assembly (Pericard *et al.*, 2018; Gruber-Vodicka *et al.*, 2020). The assembled read and the  
730 details of the command lines are provided on GitHub at  
731 <https://npollet.github.io/metatetard/Metagenome.html>).

## 732 **Metagenome assembly and gene prediction**

733 We filtered and trimmed reads according to their quality and excluded those derived from  
734 *Xenopus* genome using metaWRAP. We selected one assembly of these reads obtained with  
735 IDBA-UD using k-mers from 72 to 124 (Peng *et al.*, 2012; Uritskiy *et al.*, 2018). More details  
736 are given in the Supplementary Extended\_materials\_and\_methods. We performed binning on  
737 contigs longer than 1000 bp using maxbin, concoct, metabat and DASTool (Alneberg *et al.*,  
738 2014; Wu *et al.*, 2016; Sieber *et al.*, 2018; Kang *et al.*, 2019). We visualized binning results  
739 using vizbin (Laczny *et al.*, 2015). We mapped OTUs, metagenome and metatranscriptome  
740 reads to the assembly using BBTools to derive coverage and tpm values at the scaffold and  
741 gene levels (BBMap).

742 We predicted CDS on the assembled metagenome scaffolds using Prokka (Seemann, 2014).  
743 We used Minpath for metabolic pathway prediction following the strategy and the tools  
744 provided in the IMP pipeline scripts as described in [https://metagenomics-](https://metagenomics-workshop.readthedocs.io)  
745 [workshop.readthedocs.io](https://metagenomics-workshop.readthedocs.io) (Ye and Doak, 2009; Narayanasamy *et al.*, 2016). We mapped KEGG

746 and EC identifiers on the Interactive Pathway Explorer V3 (iPATH3) to obtain a map of the  
747 tadpole's gut microbiota metabolic pathway (Letunic *et al.*, 2008; Yamada *et al.*, 2011b)(Darzi  
748 *et al.*, 2018).

749

## 750 **List of abbreviations**

751 NF: Nieuwkoop and Faber developmental stage

## 752 **Declarations**

753 **Ethics approval and consent to participate:** We used standard rearing conditions for  
754 *Xenopus* tadpoles and adults and all animal experiments were conducted in accordance with  
755 the regulation on Animal Experimentation (registration number 91–401 provided by Direction  
756 Départementale des Services Vétérinaires de l'Essonne).

### 757 **Consent to publication:**

758 Not applicable

759 **Availability of data and material:** The sequence datasets generated and/or analyzed during  
760 the current study are available in the EMBL Nucleotide Sequence Database (ENA),  
761 metagenomic (ERS716504) and metatranscriptomic (ERS716505)  
762 <https://www.ebi.ac.uk/ena/data/view/PRJEB9311>. 16S rRNA gene sequence data are available  
763 at <https://www.ebi.ac.uk/ena/data/view/PRJEB38248>. R scripts for data analysis are provided  
764 on GitHub at <https://npollet.github.io/metatetard/>.

765 Other data type supporting the findings are available within the article and its supplementary  
766 files.

767 **Competing interests:** The authors declare that they have no competing interests.

768

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776 IDEX-0003-02, respectively).

777 **Author’s contributions:** All authors read and approved the final manuscript. TS and IC  
778 performed experimental work under the guidance of NP. MB designed and supervised TC for  
779 cytometry, and analyzed cytometry results. TC performed whole genome shotgun sequence  
780 analysis. NP performed 16S rRNA gene sequence analysis. TS and NP wrote the manuscript.

781

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791

792

793

794 **Conflict of Interest**

795 None

796 **References (100 max)**

797

798 **Figure legends**

799 **Figure 1: Enumeration of bacteria in the *Xenopus tropicalis* gut during development.**

800 **A. Cytometric profiles.** Each dot in the plot represents one event (i.e. a cell), the color  
801 represents the density of overlapping point from red to blue. The vertical axis represents DNA  
802 content by the measurement of propidium iodide (PI) fluorescence (614/20nm) and the  
803 horizontal axis represents the relative cell size by the measurement of forward scatter. Different  
804 bacterial populations are highlighted by an ellipse and identified by a text label. The top-right  
805 events labelled with a “b” correspond to the beads used for normalization. Background signals  
806 have been removed. **B. Changes of bacterial load during development.** The vertical axis  
807 represents the number of bacteria and the horizontal axis corresponds to the different stages of  
808 development. Each dot corresponds to a biological replicate, the error bar represents the  
809 standard deviation obtained by technical replicates. A ★ marks the beginning of feeding and  
810 ★★ marks the change from the tadpole to the adult diet.

811 **Figure 2: Phylum and Family-level compositional changes in the *Xenopus* gut microbiome**  
812 **across development.**

813 **A.** Bar plot representation of the bacterial diversity at the phylum level. From top to bottom,  
814 the results for the different life stages studied are shown: Premetamorphic tadpoles (NF54 to

815 NF56); Prometamorphic tadpoles (NF58 to NF61); Metamorphic tadpole (NF 62); Froglet  
816 (NF66); Adult: sexually mature adults (males and females). B. Bar plot representation of the  
817 bacterial diversity at the family level, in the same order as A. C. Same as A but viewed at the  
818 sample level to highlight the interindividual differences. D. Bar plot representation of gram  
819 positive and gram negative bacterial phyla.

820 **Figure 3: *Xenopus* microbiota across several adult organs and samples**

821 **A. Bar plot representation of the bacterial phylum diversity. B. Principal coordinate**  
822 **analysis of microbial communities across several adult organs and samples.** Community  
823 membership was analyzed using unweighted UniFrac distance, and community structure was  
824 analyzed using weighted UniFrac distance. Percentages of the explained variations are  
825 indicated on both axes.

826

827 **Figure 4: Characterization of the prevalence and abundances of active bacteria across**  
828 ***Xenopus* development from post-hatching to metamorphosis.**

829 **A. Heatmap of the prevalence and abundances for 33 OTUs.** The detection threshold scale  
830 is logarithmic and the metric used was the relative abundance. OTUs were identified using their  
831 taxonomic affiliation at the family genus or species level according to the resolution available  
832 for a given OTU. This analysis was performed on OTUs characterized by a minimal prevalence  
833 of 50 % and a minimal abundance of 0.001%.

834 **B. Abundance heatmap for the 33 most prevalent OTUs across the developmental stages**  
835 **sampled.** Two clutches were followed up across development, and the white dotted lines  
836 separate the two biological replicate samples. The legend for stages is as follows: PE is a post-  
837 hatching NF30 tailbud embryonic stage; NF41 is a premetamorphic tadpole non-feeding stage;  
838 NF48 is a premetamorphic tadpole feeding stage; NF50; NF 52 and NF56 are premetamorphic

839 tadpole feeding stages; NF60 is a prometamorphic tadpole stage; NF63 are metamorphic  
840 tadpole stages; NF66 is a froglet stage.

841 **Figure 5: Influence of the tadpole's diet on their gut microbiome.**

842 **A. Bar plot representation of the bacterial phylum diversity. B. Principal coordinate**  
843 **analysis of microbial communities across several adult organs and samples.** Community  
844 membership was analyzed using unweighted UniFrac distance, and community structure was  
845 analyzed using weighted UniFrac distance. Percentages of the explained variations are  
846 indicated on both axes. Tadpole Food 1 (D1 samples) was a micro planktonic-based diet and  
847 tadpole food 2 (D2 samples) (food 1) was a sterile flour.

848

849 **Figure 6: *X. tropicalis* microbiome transmission**

850 **A. Bar plot representation of the bacterial phylum diversity.**

851 **B. Box-plot representation of weighted UniFrac distances between eggs, skin and feces.**  
852 Letters a, b and c indicate significant differences at the 0.05 level between or within samples  
853 according to pairwise Dunn's rank sum post-hoc test (see Methods section for details). Note  
854 the outliers in the feces sample and in the Eggs-skin comparison.

855 **C. Bar plot representation of source proportions in egg microbiomes.**

856 **D. Principal coordinate analysis of microbial communities across eggs, feces and skin**  
857 **samples.** Community composition was analyzed using PhilR distance. Percentages of the  
858 explained variations are indicated on both axes.

859

860 **Figure 7: Bacterial taxonomic profiles from *X. tropicalis* prometamorphic tadpole gut. A.**

861 Histogram representation of the bacterial phylum diversity observed in a pool of five  
862 prometamorphic tadpole's guts at stage NF56. metaDNA: metagenomic analysis; metaRNA:  
863 metatranscriptomic analysis. Each color in the histogram refers to the phylum as described in

864 the legend. **B.** Vizualisation of the metagenomic assembly. Each bin identified using DASTool  
865 is represented in a different colour, each circle is a contig. The circles diameter is proportional  
866 to contig length.

867

868 **Figure 8: Overview of *X. tropicalis* tadpole's microbiota metabolic functions.** **A.** This  
869 barplot represents the read coverage (x-axis, in transcripts per kilobase million) on genes  
870 encoding enzymes participating in various Metacyc pathways (y-axis). **B.** Counts of  
871 metagenome bins, genes and scaffolds in which enzymes involved in selected biosynthetic  
872 pathways. Expressed genes encoding enzymes involved in selected KEGG modules mentioned  
873 on the y-axis were counted.

874

875 **Supplementary data R scripts: R markdown rendered html files are available at**  
876 **<https://npollet.github.io/metatetard>**

877

878 **Additional files:**

879 **Additional file 1: Supplementary\_table\_1.csv**

880 File format: csv

881 Title of data: Metadata.

882 Description of data: This file contains the metadata corresponding to samples analysed by 16S  
883 rRNA gene metabarcoding.

884

885 **Additional file 2: Supplementary\_table\_2**

886 File format: xlsx

887 Title of data: Compilation of statistical results in tables.

888 Description of data: This file contains seven tables compiling the results of significance tests  
889 for alpha and beta diversity metrics.

890

891 **Additional file 3: Figure\_S1**

892 File format: pdf

893 Title of data: Microbial cell populations identified by flow cytometry across developmental  
894 stages.

895 Description of data: Cytometric profiles obtained at different stages of tadpole's development  
896 were used to identify visually bacterial populations. Each population was plotted according to  
897 its DNA content and relative size (error bars correspond to the standard error deviation).  
898 Vertical axis represents the measurement of fluorescence (propidium iodide - 614/20nm) and  
899 the horizontal axis the measurement of relative cell size (forward scatter, in arbitrary units).  
900 The bacterial populations clearly corresponding across life stages are grouped by a dotted  
901 ellipse.

902

903

904 **Additional file 4: Figure\_S2**

905 File format: pdf

906 Title of data: Rarefaction and species accumulation curves.

907 Description of data: These graphs present rarefaction curves (top) and species accumulation  
908 curves (bottom) for the different samples used in the listed experiments. \* For premetamorph  
909 samples, the rarefaction curve is shown only up to 60,000 reads for the sake of consistency  
910 between all plots. Species richness corresponds to OTUs number. A detailed analysis is  
911 presented at [https://npollet.github.io/metatetard/xpall\\_rarefaction\\_phyloseq.html](https://npollet.github.io/metatetard/xpall_rarefaction_phyloseq.html)

912

913

914

915 **Additional file 5: Figure\_S3**

916 File format: pdf

917 Title of data: Bacterial communities in the *Xenopus* gut across development.

918 Description of data:

919 A. Graphical representation of the bacterial species richness. Each point corresponds to the  
920 breakaway richness estimate, error bars represent the measurement error according to the  
921 breakaway model. Premet: premetamorphic tadpoles NF54 to NF56. Promet: prometamorphic  
922 tadpoles NF58 to NF61; Meta: Metamorphic tadpole NF 62; Fro: Froglet NF66; Adult: sexually  
923 mature adults. Horizontal lines represent significant differences between the life stages  
924 connected;  $p=0.000$  both between Premet. and Promet. and between Premet. and Adult. B.  
925 Representation of the bacterial species diversity measured using the Shannon index. Each large  
926 point corresponds to the divnet Shannon estimate from the samples of the same life stage, error  
927 bars represent the measurement error. Each small point corresponds to the divnet Shannon  
928 estimate from a given sample. The error bar corresponding to the measurement error can not be  
929 seen at this scale. Horizontal lines represent significant differences between the life stages  
930 connected;  $p=0.000$  in all significant cases including the global test ( $p=0.875$  for Premet-Meta).  
931 C. Principal coordinate analysis of gut-associated microbial communities during *Xenopus*  
932 development. Community membership was analyzed using unweighted Unifrac distance, and  
933 community composition was analyzed using philR distance. Percentages of the explained  
934 variations are indicated on both axes.

935

936

937 **Additional file 6: Figure\_S4**

938 File format: pdf

939 Title of data: Shared and contrasting bacterial communities in the *Xenopus* gut microbiome  
940 across development.

941 Description of data: A : Venn diagram showing the number of shared OTUs between samples  
942 of the same developmental stage category. B : Bar plot of the total number of OTUs in each  
943 developmental stage category. C : Number of shared and specific OTUs. D : Heatmap plot of  
944 the prevalence and abundances for low-abundance OTUs. The detection threshold scale is  
945 logarithmic and the metric used was the relative abundance. This analysis was performed on  
946 OTUs characterized by a minimal prevalence of 50 % and a minimal abundance of 1e-5 %. E.  
947 Prevalence and abundances of the most common and the most abundant OTUs across  
948 development. OTUs were identified using their taxonomic affiliation at the family genus or  
949 species level according to the resolution available for a given OTU. This analysis was  
950 performed on OTUs characterized by a minimal prevalence of 50 % and a minimal abundance  
951 of 0.001%. The right panel shows an abundance heatmap for the same OTUs to visualize their  
952 abundances in each sample.

953

954 **Additional file 7: Figure\_S5**

955 File format: pdf

956 Title of data: Contrasting OTUs across development

957 Description of data: A : Abundance fold changes across development. Contrasting OTUs were  
958 identified by filtering OTUs with an abundance of at least 100 reads across 20% of sample and  
959 using DEseq2 with a significance cutoff of 0.01. B : Heatmap representation of contrasting  
960 OTUs abundances across development. The heatmap was produced using a principal  
961 component analysis based on UniFrac distances.

962



963 **Additional file 8: Figure\_S6**

964 File format: pdf

965 Title of data: Variations of the phylogenetic structure of the *Xenopus* gut microbiota during  
966 development.

967 Description of data: Standardized effect sizes of the Mean pairwise distance (left panel) and of  
968 the mean nearest taxon distance (right panel) measured using Faith' PD index for *X. tropicalis*  
969 gut microbial communities across developmental life stages as indicated on the x-axis. Letters  
970 a, b and c indicate significant differences at the 0.05 level between developmental stages  
971 according to pairwise Dunn's rank sum post-hoc test (see Methods section for details).

972

973 **Additional file 9: Figure\_S7**

974 File format: pdf

975 Title of data: *Xenopus* tadpole active bacterial communities: alpha-diversity, bacterial phylum  
976 diversity and source proportions

977 Description of data: A : Bar plot representation of OTU richness and phylogenetic diversity in  
978 active bacterial communities. Two clutches were followed up across early development, and  
979 the light and dark grey bars correspond to the two biological replicate samples. The legend for  
980 stages is as follows: NF30 is a post-hatching tailbud embryonic stage; NF41 is a  
981 premetamorphic tadpole non-feeding stage; NF48 is a premetamorphic tadpole feeding stage;  
982 NF50 and NF 52 are premetamorphic tadpole feeding stages. B : Bar plot representation of the  
983 bacterial phylum diversity. C : Bar plot representation of source proportions in froglet gut  
984 microbiome.

985

986 **Additional file 10: Figure\_S8**

987 File format: pdf

988 Title of data: Impact of the food regime on tadpole growth and development.

989 Description of data: A: Impact of food diet on tadpole growth and development. Tadpoles were  
990 fed using either a micro planktonic-based diet (food 1) or a sterile flour (food 2). Their growth  
991 was assessed by weighting them, and their development was monitored by assessing their  
992 developmental stage according to the Nieuwkoop and Faber developmental table. The food  
993 regime did not change significantly tadpole growth (Kruskal-Wallis chi-squared = 0.99, df = 1,  
994  $p = 0.32$ ) or development (Kruskal-Wallis chi-squared = 0.34, df = 1,  $p = 0.56$ ). B : Impact of  
995 the food regime on tadpole's guts microbial communities. These bar plot shows values of the  
996 Standardized effect size of the Mean Pairwise Distance (SES MPD) and of the Mean Nearest  
997 Taxon Distance (SES MNTD) according to the food regime given to groups of tadpoles. The  
998 result of a Wilcoxon signed-rank test is shown above the graphs, with  $W$  being the Wilcoxon  
999 test statistic computed by this test,  $p$  the p-value,  $r$  the effect size, CI the confidence interval of  
1000 the effect size and  $n$  the number of observations (see  
1001 <https://indrajeetpatil.github.io/statsExpressions/>). C : Abundance ratios of the OTUs found  
1002 significantly different according to a change of diet.

1003

1004 **Additional file 11: Figure\_S9**

1005 File format: html

1006 Title of data: Krona plot view of taxonomic affiliations inferred from a MATAM assembly of  
1007 *X. tropicalis* tadpole's gut metagenomic reads.

1008 Description of data: *X. tropicalis* tadpole's gut metagenomic reads were used as input for the  
1009 identification and assembly of SSU rRNA sequences using MATAM. Taxonomic affiliations  
1010 were then inferred using SILVA

1011

1012 **Additional file 12: Figure\_S10**

1013 File format: html

1014 Title of data: Results of a phyloFlash assembly of *X. tropicalis* tadpole's gut metagenomic  
1015 reads.

1016 Description of data: *X. tropicalis* tadpole's gut metagenomic reads were used as input for the  
1017 identification and assembly of LSU and SSU rRNA sequences using phyloFlash. The results  
1018 for Spades and Emirge assembly engines are shown. Taxonomic affiliations were then inferred  
1019 using SILVA

1020

1021 **Additional file 13: Figure\_S11**

1022 File format: html

1023 Title of data: Krona plot view of taxonomic affiliations inferred from a MATAM assembly of  
1024 *X. tropicalis* tadpole's gut metatranscriptomic reads.

1025 Description of data: *X. tropicalis* tadpole's gut metatranscriptomic reads were used as input for  
1026 the identification and assembly of SSU rRNA sequences using MATAM. Taxonomic  
1027 affiliations were then inferred using SILVA

1028

1029 **Additional file 14: Figure\_S12**

1030 File format: html

1031 Title of data: Results of a phyloFlash assembly of *X. tropicalis* tadpole's gut metatranscriptomic  
1032 reads.

1033 Description of data: *X. tropicalis* tadpole's gut metatranscriptomic reads were used as input for  
1034 the identification and assembly of LSU and SSU rRNA sequences using phyloFlash. The results  
1035 for Spades, Emirge and Trinity (trusted contigs) assembly engines are shown. Taxonomic  
1036 affiliations were then inferred using SILVA

1037

1038 **Additional file 13: Supplementary table 3**

1039 File format: xlsx

1040 Title of data: Comparison of metagenomic assemblies

1041 Description of data: This file contains the metrics obtained using metaQUAST on different  
1042 assemblies of metagenomic reads.

1043

1044 **Additional file 14: Supplementary table 4**

1045 File format: xlsx

1046 Title of data: Results of DASTool binning

1047 Description of data: This file contains the summary of DASTool binning results.

1048

1049 **Additional file 15: Figure\_S13**

1050 File format: html

1051 Title of data: Krona plot view of predicted KEGG metabolic pathways.

1052 Description of data: KEGG metabolic pathways were predicted based on Minpath inference  
1053 from EC enzyme numbers obtained using prokka.

1054

1055

1056 **Additional file 16: Figure\_S14**

1057 File format: html

1058 Title of data: Krona plot view of predicted Metacyc metabolic pathways.

1059 Description of data: Metacyc metabolic pathways were predicted based on Minpath inference  
1060 from EC enzyme numbers obtained using prokka.

1061

1062 **Additional file 17: Figure\_S15**

1063 File format: pdf

1064 Title of data: Metabolic map of *X. tropicalis* genome and its gut metagenome.

1065 Description of data: This metabolic pathway highlights in green the metabolic pathways  
1066 predicted from the *Xenopus* genome and in blue those predicted from the tadpole gut  
1067 metagenome. This map can be interactively accessed at  
1068 <https://pathways.embl.de/selection/pWbci4bo871W8Qm8XKF>

1069

1070

1071 **Additional file 18: Figure\_S16**

1072 File format: pdf

1073 Title of data: Selected cases of metabolic potential of *X. tropicalis* genome and its gut  
1074 metagenome.

1075 Description of data: Fragments of biosynthetic pathways for common short-chain fatty acids,  
1076 nitrogen recycling and B-vitamins is depicted.

1077 This metabolic pathway highlights in green the metabolic pathways predicted from the *Xenopus*  
1078 genome and in blue those predicted from the tadpole gut metagenome. This map can be  
1079 interactively accessed at <https://pathways.embl.de/selection/pWbci4bo871W8Qm8XKF>

1080

1081 **Additional file 19: Extended\_materials\_and\_methods**

1082 File format: docx

1083 Title of data: Extended materials and methods.

1084 Description of data: This file contains an extended version of the materials and methods

1085