Microbiome turnover during offspring development varies with maternal care, but not moult, in a hemimetabolous insect

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Abstract

The ecological success of insects often depends on their association with beneficial microbes. However, insect development involves repeated moults, which can have dramatic effects on their microbial communities. Here, we investigated whether and how moulting affects the microbiome of a hemimetabolous insect, and whether maternal care can modulate these effects. We reared European earwig juveniles with or without mothers and used 16S rRNA metabarcoding to analyse the whole microbiome of eggs, recently and old moulted individuals at four developmental stages and the resulting adults. The 218 samples obtained showed that the microbiome diversity changed non-linearly during development and that these changes were associated with bacterial biomarkers. Surprisingly, these changes did not occur during moulting, but rather between the beginning and end of certain developmental stages. We also found that access to maternal care affected the microbiome of both juveniles and adults, even when the last contact with mothers was two months before adulthood. Overall, these results provide new insights into our understanding of the (in)stability of the microbiome in hemimetabolous insects and its independence from moult. More generally, they question the role of microbiome acquisition through maternal care in maintaining family life in species where this behaviour is facultative.

Keywords: Microbiome, Horizontal transmission, Vertical transmission, Metamorphosis, Sociality

Introduction

Insects are the most diverse and abundant animal taxon on Earth, comprising more than half of the animal kingdom (Mora *et al.*, 2011; Berenbaum, 2017; Samways, 2018). One reason for their evolutionary success is their frequent association with a large and complex diversity of beneficial microorganisms (Shapira, 2016; Sudakaran *et al.*, 2017). This is because this association can help their host colonize new ecological niches and mediate insect speciation and adaptation to a variety of environments (Berlanga and Guerrero, 2016; Shapira, 2016). However, maintaining these associations is challenging for insects, as it requires hosts to retain their microbes despite the numerous and successive moults (or ecdysis) that occur between the egg and adult stages (Bright and Bulgheresi, 2010). From a microbial perspective, these moults are risky as they can lead to a partial purge from the host, inducing a microbial bottleneck in which almost all symbionts are lost (McFrederick *et al.*, 2014; Zhukova *et al.*, 2017). Moults can also lead to critical changes in the habitat used by microbes within the host, which in turn can rapidly induce major turnover in their communities (Engel and Moran, 2013). For the hosts, moulting also poses a significant challenge, as they need to retain or (re)acquire the beneficial symbionts (Arce *et al.*, 2012; Wang and Rozen, 2017) while coping with surrounding pathogenic microorganisms susceptible to take their place in the host (Salem *et al.*, 2015; Hammer and Moran, 2019). Therefore, it is necessary for both host insects and beneficial microorganisms to develop strategies to ensure the continuity of their association throughout host development.

To ensure the (re)inoculation and maintenance of beneficial microorganisms throughout development, insects can adopt two non-mutually exclusive strategies. On one hand, they can acquire these microorganisms from direct contact with their

environment and conspecifics, a process called horizontal transmission. This process can be particularly important when individuals live in groups, share a nesting environment and/or when moulting leads to the loss of these microorganisms at each development stage (Raina *et al.*, 2008; Nalepa, 2020; Rose *et al.*, 2023). On the other hand, hosts can acquire beneficial microorganisms from their parents, a process called vertical transmission (Bright and Bulgheresi, 2010; Sachs *et al.*, 2011; Hosokawa and Fukatsu, 2020; Michaud *et al.*, 2020). In insects, vertical transmission was long thought to occur mainly through the transfer of microorganisms directly into the eggs, which the resulting offspring then had to maintain throughout their development. However, many insect parents provide care to their eggs and juveniles after oviposition (Meunier *et al.*, 2022) and recent studies show that this care can also mediate a vertical transmission of microorganisms. For instance, mothers can deposit external secretions containing symbionts on the eggshell, transfer symbionts to their juveniles through mouth-to-mouth contact (trophallaxis) during family life, and juveniles can acquire these symbionts soon after hatching by ingesting their parents' feces (Klass *et al.*, 2008; Lombardo, 2008; Bright and Bulgheresi, 2010; Powell *et al.*, 2014; Zhukova *et al.*, 2017; Onchuru *et al.*, 2018). Access to maternal care can thus ensure the acquisition and reacquisition of beneficial microbes by moulting juveniles, thus possibly strengthening the stability and evolutionary trajectory of symbiotic associations.

While our current understanding of the consequences of moulting and maternal care on the dynamics of the host microbiome during development is mainly based on holometabolous insects, little is known about these consequences on hemimetabolous species (Hammer and Moran, 2019; Girard *et al.*, 2023). The focus on holometabolous species is explained by the fact that their immature stages have a morphology and sometimes an ecology very different from those of adults, which raises obvious

81 questions about the fate of their microbiome during metamorphosis (Johnston *et al.*,
82 2019). In contrast, hemimetabolous insects have immature stages called nymphs that
83 are very similar in morphology and ecology to the adult, and their juveniles undergo
84 only gradual morphological changes through successive moults (Johnston *et al.*,
85 2019). The impact of these gradual changes on the dynamics of the host microbiome
86 has received comparatively much less attention, and a few studies suggest that it may
87 be stable throughout development such as in Blattodea, Orthoptera, and Hemiptera
88 (Sudakaran *et al.*, 2012; Manthey *et al.*, 2022). However, it remains unclear whether
89 this stability is universal across species and, importantly, whether it is due either to a
90 non-purging effect of moulting on microbial communities, the fact that host microbial
91 niches do not change during development and thus select for the same microbial
92 communities, and/or that maternal care ensures the maintenance of the microbial
93 community through vertical transmission.

94 In this study, we investigated whether and how the microbiome of the
95 hemimetabolous European earwig *Forficula auricularia* L. (Order Dermaptera:
96 Forficulidae) changes during juvenile development and tested whether these potential
97 changes were due to moulting events, stage-specific microbial niches and/or offspring
98 access to maternal care. In this species, females oviposit in individual burrows in early
99 winter (Meunier *et al.*, 2012; Tourneur and Meunier, 2020) after which they stop
100 foraging and provide extensive forms of care, including egg grooming (Boos *et al.*,
101 2014; Diehl and Meunier, 2018). About 50 days later, the eggs hatch and the mothers
102 stay with their juveniles for about two more weeks. During this time, they continue to
103 provide care to their nymphs, such as allo-grooming and food provisioning (Lamb,
104 1976; Kölliker, 2007). Mothers leave the nest shortly after the nymphs have moulted
105 for the second time (the first moult occurs at the time of hatching), and the nymphs

then moult three more times before reaching adulthood two months later (Thesing *et al.*, 2015; Tourneur *et al.*, 2020). Whether the offspring microbiome changes during development and whether maternal care influences these changes are unknown in the European earwig. However, the microbiome of the eggshell is known to change over 16 days and to be partly influenced by maternal presence in the maritime earwig *Anisolabis maritima* (Greer *et al.*, 2020).

Here, we reared 20 families of the European earwig from egg to adult stages. Fifteen families were reared with their mothers for the first 15 days after hatching (*i.e.*, the normal duration of family life), while five families were reared without mothers from egg hatching onward. This was possible as post-hatching maternal care is facultative in this species (Thesing *et al.*, 2015). We then used 16S rRNA metabarcoding to analyse the whole microbiome of the eggs, both the freshly and old moulted nymphs of each instar and finally, the resulting adult offspring (males and females) of all these families. Overall, we found that the microbiome of earwig offspring surprisingly changed during development. We also showed that these changes are not due to a purging event during moulting, but rather likely reflect stage-specific microbial niches in the nymphs. Finally, we found that access to maternal care has both short- and long-term effects on the microbiome of offspring.

Material and methods

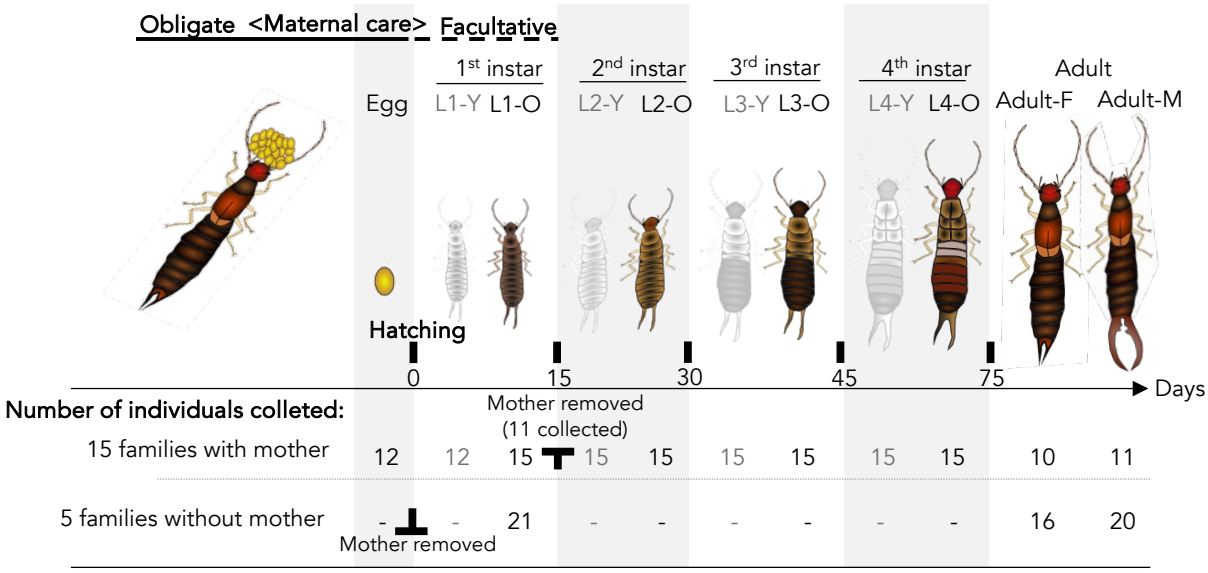
Earwig sampling and laboratory rearing

The eggs, nymphs and adults analysed in this study were the first-generation progeny of 20 females of *F. auricularia* sp "A" (Wirth *et al.*, 1998; González-Miguéns *et al.*, 2020). These 20 females were part of a large field sampling of earwig males and

131 females conducted in an orchard near Valence, France (44°58′38″N, 4°55′43″E) in the
132 summer of 2021. Just after field sampling, these individuals were randomly distributed
133 into plastic containers containing 100 females and 100 males and then maintained
134 under standard laboratory conditions to allow for uncontrolled mating (Koch and
135 Meunier, 2014; Sandrin *et al.*, 2015). In November 2021, each female was isolated to
136 mimic her natural dispersal from the groups and to stimulate oviposition (Kölliker,
137 2007). These females were transferred to individual Petri dishes (55 mm x 12 mm)
138 lined with moist sand (Körner *et al.*, 2018) and maintained in constant darkness at 10°C
139 until oviposition and egg hatching. From isolation until oviposition, females were fed
140 ad libitum with a laboratory-prepared food consisting mainly of carrots, cat food, seeds
141 and pollen (Kramer *et al.*, 2015). Food was renewed each week, but removed from the
142 day of oviposition until the day of egg hatching, as this is when the mothers typically
143 stop their foraging activity (Kölliker, 2007; Van Meyel and Meunier, 2020). From this
144 large pool of females, we haphazardly selected 20 clutches in which the mothers
145 produced 50 eggs (clutch size typically varies from 30 to 60 eggs, Tourneur and
146 Gingras, 1992) for our measurements. The remaining females were used in other
147 experiments not presented here.

148 On the day of egg hatching, the 20 selected clutches were transferred to larger
149 Petri dishes (145 mm x 13 mm) lined with moist sand (Körner *et al.*, 2018) to manipulate
150 maternal presence during offspring development. Of these 20 clutches, five (randomly
151 selected) had their mothers removed to subsequently prevent post-hatching maternal
152 care and any mother-offspring interactions. For the remaining 15 clutches, the nymphs
153 were kept with their mother for 14 days after egg hatching (which is the natural length
154 of family life in this species) and then separated from their mother for the rest of their
155 development. All clutches were maintained under laboratory conditions at 18-20°C

156 under a 12:12 light:dark photoperiod, and received the laboratory-prepared food twice
157 a week (see above).
158
159 **Experimental design and sample collection**
160 Overall, we analysed the microbiome of 218 samples collected throughout earwig
161 development (Figure 1, Table S1).
162



163

164 **Figure 1: Overview of the experimental design.** All developmental stages from eggs
165 to adults were sampled for microbiome analysis before (coloured specimens) and after
166 (white specimens) each moult. Note that the L0 developmental stage was not sampled
167 as it moults during egg hatching (Tourneur *et al.*, 2020). Although it is facultative, post-
168 hatching maternal care usually continues until the nymphs reach the 2nd instar. We
169 collected a total of 150 samples across all developmental stages in families with post-
170 hatching maternal care, and 58 samples from the 1st instar and adults in families
171 without maternal care.
172
173 As ecdysis is known to induce a microbial shift in many insect species, we sampled all
174 developmental stages (except eggs and adults) both immediately after the moult
175 (freshly moulted) and several days after the moult (old moulted). Freshly moulted

176 nymphs have a white (compared to dark) colour, which typically lasts for maximum 3h
177 in early instar nymphs and up to 6h in late instar (MC Cheutin, pers. obs.). For the 15
178 clutches with maternal care, we obtained a total of 12 egg samples (Egg), 37 first instar
179 nymphs of which 12 were freshly moulted (later called L1-Y) and 15 were old moulted
180 (L1-O), 30 second instar nymphs of which 15 were freshly moulted (L2-Y) and 15 were
181 old moulted (L2-O), 30 third instar nymphs of which 15 were freshly moulted (L3-Y)
182 and 15 were old moulted (L3-O), 30 fourth instar nymphs of which 15 were freshly
183 moulted (L4-Y) and 15 were old moulted (L4-O), and finally 32 adults of which 11 field-
184 sampled mothers (Mother), 10 adult female offspring (Adult-F) and 11 adult male
185 offspring (Adult-M). For the 5 clutches without maternal care, we only used a total of
186 21 old moulted first instar nymphs, 16 adult females and 20 adult males as we were
187 only interested in the short and long-term effect of maternal presence/absence on
188 offspring s (Figure 1, see details in Table S1). Note that the first observable
189 developmental stage of the nymphs is called L1, as the moult that occurs at hatching
190 is called L0 in this species (Tourneur *et al.*, 2020). Moreover, the sex of an individual
191 can only be determined in adults, where the shape of the forceps is straight in females
192 and curved in males. Each sample was collected individually and immediately
193 transferred to Eppendorf tubes at -80°C until DNA extraction.
194

195 **Genomic extraction and 16S amplification**

196 Total genomic DNA was extracted using the NucleoMag® Tissue extraction kit
197 (Macherey-Nagel™, Düren, Germany) and the V3-V4 region of the 16S rDNA gene
198 was amplified with the prokaryotic primers 343F (5′- ACGGRAGGCAGCAG – 3′) and
199 784R (5′- TACCAGGGTATCTAATC – 3′) (Muyzer *et al.*, 1993) coupled with platform-
200 specific Illumina linkers. We performed PCR reactions using the Taq Polymerase

201 Phusion® High-Fidelity PCR Master Mix with GC buffer and prepared them according
202 to the manufacturer's instructions (Qiagen, Hilder, Germany). PCR amplification steps
203 involved an initial denaturing step for 30 sec at 98°C, followed by 22 amplification
204 cycles (denaturation for 10 sec at 98°C; annealing for 30 sec at 61.5°C; extension for
205 30 sec at 72°C), and ended by a final extension step of 10 min at 72°C. Electrophoresis
206 migration was run to check the probe specificity and amplification success. Extraction
207 and amplification steps involved several blank controls to confirm that samples were
208 not contaminated by environmental microorganisms. Samples were amplified in
209 duplicates and equally pooled for a final product of 30 µL further sequenced with
210 2x250bp Illumina MiSeq technology at the Bio-Environnement platform (University of
211 Perpignan, France). As the blanks were all negative, we did not send them for
212 sequencing.

213

214 **Bioinformatic process**

215 The obtained libraries were trimmed and filtered using the quality profiles from the
216 DADA2 algorithm v1.24.0 (Callahan *et al.*, 2016), cleaned for errors, dereplicated and
217 inferred towards Amplicon Sequence Variants (ASVs) (Glassman and Martiny, 2018).
218 Chimeras were removed and taxonomy assignment was performed on a count table
219 where forward and reverse ASVs were merged and pooled, using the SILVA reference
220 database (release 138) (Quast *et al.*, 2012). The multiple alignment of the sequences
221 was provided with the MAFFT program (Katoh, 2002) and we inferred the phylogenetic
222 tree with the FastTree 2 tool (Price *et al.*, 2010) and Phangorn package v2.8.1 (Schliep,
223 2011). The table was then transformed into a phyloseq object using the phyloseq
224 package v1.40.0 (McMurdie and Holmes, 2013) of which we removed the 529
225 sequences of mitochondrial origin and the 30 800 unknown sequences. As the 2 700

226 Eukarya sequences had no affiliation at Phylum level and were sparsely distributed,
227 they were also removed. Finally, we obtained a final dataset constituted by 4 163 989
228 sequences (3 780 ASVs) ranging from 3 570 to 32 946 sequences/sample.

229

230 **Identification of the core microbiomes**

231 We defined the core microbiome as the set of microbial taxa (*i.e.,* ASVs) that are
232 characteristic of all samples. We obtained this core microbiome using the species
233 abundance distribution (SAD) patterns of each ASVs (Magurran and Henderson,
234 2003). This approach is frequently used in the literature (Fillol *et al.*, 2016; Jeanbille *et*
235 *al.*, 2016; Cheutin *et al.*, 2021; Neu *et al.*, 2021) and allows us to distinguish between
236 core and satellite ASVs, while avoiding the use of subjective and arbitrary occurrence
237 and abundance thresholds (Magurran and Henderson, 2003). We first calculated an
238 index of dispersion (*i.e.,* the variance to mean ratio, VMR) for each ASV within each
239 developmental stage. We then tested whether these indices followed a Poisson
240 distribution, falling between the 2.5 and 97.5% confidence limits of the Chi^2 distribution
241 (Krebs, 1999). ASVs with index values outside these confidence limits were considered
242 as part of the core microbiome, while the others were considered as satellite ASVs.
243 This process provided us with stage-dependent core microbiomes for the eggs, each
244 instar nymph stages, adult offspring and mothers, and we finally merged all these cores
245 to obtain a global core microbiome (Figure S1).

246

247 **Functional predictions of the core microbiomes**

248 We used the PICRUST2 algorithm (Douglas *et al.*, 2020) to predict the potential
249 functions associated with each of the core microbiomes generated above. In brief, the

250 tool inserts each ASV sequence into a reference tree (EPA-ng, Barbera *et al.*, 2019)
251 using a hidden-state prediction (HSP, castor, Louca and Doebeli, 2018) and infers a
252 KEGG ortholog function (later named KOs) based on the functional profiles obtained
253 with the nearest-sequenced taxon (MinPath, Ye and Doak, 2009). The functional table
254 counts with KOs obtained for each sample are count-normalized for the ASVs copy
255 counts and multiplied by the gene content prediction resulting from the HSP algorithm.
256 Finally, a table with three levels of KEGG pathways is constructed according to the
257 MetaCyc database v27.1 (Caspi *et al.*, 2020).

258

259 **Evolution of bacterial diversity during offspring development**

260 To test how the microbiomes of offspring changed throughout host development, we
261 calculated the alpha and beta diversities of each sample using qualitative and
262 quantitative indices for both taxonomic and phylogenetic diversity. Regarding alpha
263 diversity, which was only calculated for ASVs (not for functions), we normalized all
264 samples at the minimum sampling size of sequences per individual and checked their
265 accuracy with rarefaction curves (Cameron *et al.*, 2021; Figure S2). For alpha diversity
266 proxies, we used direct observed richness for the qualitative taxonomic richness and
267 the Shannon entropy for its quantitative equivalent. We calculated their phylogenetic
268 equivalents with the Faith and the Allen's indices (Chao *et al.*, 2010). For beta diversity,
269 we calculated the relative abundance of each ASV and KOs by sample, and we
270 calculated the Jaccard distance between samples with ASVs (and KOs)
271 presence/absence and its quantitative equivalent Bray-Curtis. For ASVs, we also used
272 their phylogenetic equivalents with the Unifrac metrics for qualitative (unweighted) and
273 quantitative (weighted) distances (Chao *et al.*, 2010; Yang *et al.*, 2021).

274 To identify which microbes and/or potential functions, if any, are specific to each
275 developmental stage, we assessed the differential abundance of ASVs and KOs with
276 a negative binomial Wald test using the DESeq2 package (Love *et al.*, 2014). In this
277 model, we entered the developmental stage, which includes all stages with young- and
278 old-nymphs. Results are presented under heatmaps using the package *pheatmap*
279 v1.0.12 where ASVs are merged by Genera and KOs are merged by KEGG pathway
280 names (third level).

281

282 **Impact of maternal presence on offspring bacterial diversity**

283 Finally, we tested whether maternal presence influenced the microbiome of their
284 offspring at both the first developmental stage and the adult stage. To this end, we
285 repeated the analyses described above using the 5 families in which the mothers were
286 removed at egg hatching and the 15 families in which the mothers remained with their
287 nymphs for 15 days after hatching (*i.e.,* until the end of family life). We draw volcano
288 plots with the package *EnhancedVolcano* v1.14.0 to contrast discriminants between
289 the presence or absence of the mother.

290

291 **Statistics**

292 We first tested the effect of developmental stage and moult (fresh versus old moult) on
293 the microbiome diversity of offspring from the 15 families in which nymphs were kept
294 with a mother during family life. For alpha diversity, we conducted four linear mixed
295 models (LMM) in which we entered each of the four microbial alpha diversity indices
296 as a response variable, the sampling time (Egg, L1-Y, L1-O, L2-Y, L2-O, L3-Y, L3-O,
297 L4-Y, L4-O, Adult-F, Adult-M and Mother) as the explanatory variable and the clutch

298 used as a random effect to control for non-independence of the biological samples. We
299 did not use a classical 2-way ANOVA approach because the two levels of moult were
300 not available for each developmental stage (*i.e.,* eggs and adults). We conducted
301 pairwise comparisons between each sampling time using the estimated marginal
302 means of the models, with P values corrected for multiple testing using Tukey methods
303 with the *emmeans* package (Lenth, 2022). For each pairwise comparison, we also
304 calculated R^2 using the *MuMIn* package (Bartoń, 2022). For beta diversity, the
305 dissimilarity based on the ASVs and KOs assemblies between each sample was first
306 illustrated in a two-dimensional Principal Coordinates Analyses (PCoA). We then
307 performed Permutational Analyses of Variances (PERMANOVAs) to test the effect of
308 the clutch (as randomized block), the sampling stage and the sex on each distance
309 matrices based on bacterial composition (*i.e.,* Jaccard, Bray-Curtis, weighted and
310 unweighted Unifrac) and on functional predictions (*i.e.*, Jaccard and Bray-Curtis). Post-
311 hoc pairwise tests between stages were performed for each dissimilarity matrix with
312 the package *pairwiseAdonis* v0.4.1 (Martinez Arbizu, 2017). These pairwise
313 comparisons allowed us to address five questions, namely whether (1) microbiome
314 diversity changes between each successive development stage (*i.e.,* Egg *vs* L1-Y, L1-
315 Y *vs* L1-O, L1-O *vs* L2-Y, etc), (2) moulting causes a shift in microbiome diversity
316 between two successive developmental stages (*i.e.,* Egg *vs* L1-Y, L1-O *vs* L2-Y, etc),
317 (3) old nymphs exhibit an instar-specific microbiome diversity (*i.e.,* Egg *vs* L1-O, L1-O
318 *vs* L2-O, L2-O *vs* L3-O, etc), (4) freshly moulted nymphs exhibit an instar-specific
319 microbiome diversity (*i.e.,* L1-Y *vs* L2-Y, L2-Y *vs* L3-Y, etc) and finally, whether (5)
320 adults offspring exhibit a sex-specific microbiome diversity (Adult-M *vs* Adult-F).

321 We tested whether maternal presence had short and/or long-term effects on the
322 microbiome diversity of their offspring using the 15 families with mothers and the 5

323 families without mothers. For alpha diversity, we conducted four LMM in which we
324 entered each of the index values as a response variable, as well as maternal presence
325 (yes or no), the developmental instar (L1-O or adult) and the interaction between these
326 two factors as explanatory variables. When the latter was non-significant, we removed
327 it after model simplification by AIC comparison. We also entered the clutch as a random
328 effect. For beta diversity, we repeated the approach detailed above by testing the effect
329 of the clutch and the presence/absence of a mother in interaction with the stage (L1-O
330 or adult), on both microbial and functional composition.

331 We performed all statistical analyses using R v4.2.0 (R Core Team, 2022). We
332 checked all model assumptions with the *DHARMa* package (Hartig, 2022). We verified
333 variance homoscedasticity between groups by comparing the distance dispersion
334 within group with the *betadisper* function (all $P > 0.05$).

335

336 Results

337 Microbiome changes during offspring development (with mothers)

338 *Description of the core microbiome*

339 In the pool of the 218 earwig samples, we detected a total of 915 ASVs core (24.21%
340 of the initial ASVs diversity), which encompasses 97.67% of the complete sequence
341 dataset (Figure S1, Table S2). This core microbiome consisted mainly of
342 Proteobacteria (52.5%), Firmicutes (26.8%), Bacteroidota (13.9%) and
343 Actinobacteriota (6.6%). They were distributed among 66 bacterial families, of which
344 Lactobacillaceae (Phylum Firmicutes - 23.5%) and Enterobacteriaceae (Phylum
345 Proteobacteria - 15.6%) were the most abundant. The abundance of the other bacterial
346 families depended on the developmental stage of the host (Figure 2).

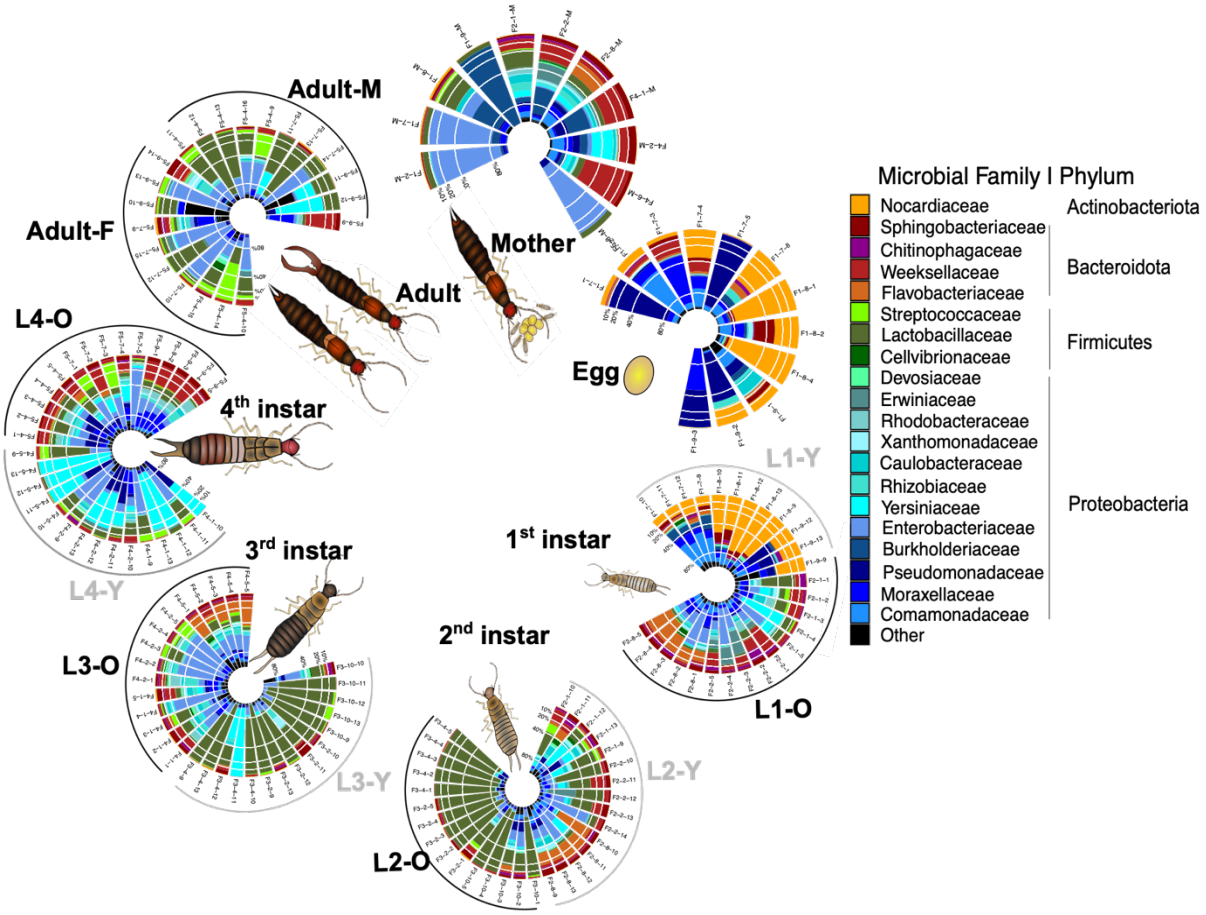


Figure 2: Composition of the European earwig core microbiome. Individual core microbiome at family scale, ordered by relative importance, grouped, and coloured by bacterial family. Specimens are ordered according to their developmental stage with freshly moulted young nymphs (grey) on one side of the circle, and old nymphs (black) on the other side.

For instance, the families Nocardiaceae (Phylum Actinobacteriota) and Comamonadaceae (Phylum Proteobacteria) dominated only the egg stage (27.6% and 13.2% respectively) and the newly hatched nymphs N1-Y (39.2% and 16.9% respectively). On a finer scale, 50 ASVs were present at all developmental stages, a number that increased to 142 ASVs when eggs were excluded (Figure S3A). The 50 common ASVs covered 29 genera, represented mainly by *Chryseobacterium, Sphingobacterium* (Phylum Bacteroidota), *Smaragdicoccus* (Phylum Actinobacterioa),

360 *Kosakonia, Stenotrophomonas*, *Paracoccus, Pseudomonas* and *Acinetobacter*
361 (Phylum Proteobacteria) (Figure S3B). Only a few ASVs were stage specific. For
362 instance, 2 ASVs (ASV66|*Pseudomonas* and ASV482|*Sphingobacterium*) were
363 specific to the eggs, 52 to the nymphs and 26 to the adults (Figure S3A).

364

365 *Changes in alpha and beta diversity during offspring development*

366 The alpha diversity of the earwig gut microbiome changed during the development of
367 the offspring (Figure 3A; Figure S4; Shannon index, Likelihood Ratio $\chi^2_{11} = 59.70$, P =
368 10^{-8} and $P < 10^{-10}$ for all other alpha diversity indices). Markedly, the nature of these
369 changes did not follow a linear or systematic pattern. Alpha diversity did not change
370 between eggs and freshly moulted 1st instar nymphs (pairwise comparisons using log-
371 rank test contrasts for Shannon index; Egg *vs* L1-Y, P = 0.921), nor did it change during
372 the entire 1st instar (L1-Y *vs* L1-O, P = 0.621). However, there was a gradual increase
373 in alpha diversity during this time, as it was greater at the end of the 1st instar than in
374 the eggs (Egg *vs* L1-O, P = 0.052). While this alpha diversity remained at a high level
375 just after the moult into 2nd instar (L1-O *vs* L2-Y, P = 1.000), it then dropped to the
376 lowest level at the end of the 2nd instar (L2-Y *vs* L2-O, *P* = 0.029). This level remained
377 low just after the moult into 3rd instar (L2-O *vs* L3-Y, P = 0.998), but then increased to
378 its highest level at the end of the 3rd instar (L3-Y *vs* L3-O, P = 0.010). Alpha diversity
379 decreased slightly just after the moult into 4th instar (L3-O *vs* L4-Y, P = 0.086, but other
380 indices are significant for this contrast) to finally remain stable until the end of the 4th
381 instar (L4-Y *vs* L4-O, P = 0.702) and in the following adult females (L4-O *vs* Adult-F, P
382 = 0.496). By contrast, it decreased slightly between the end of the 4th instar and the
383 following adult males (L4-O *vs* Adult-M, P = 0.055, but other indices are significant for
384 this contrast).

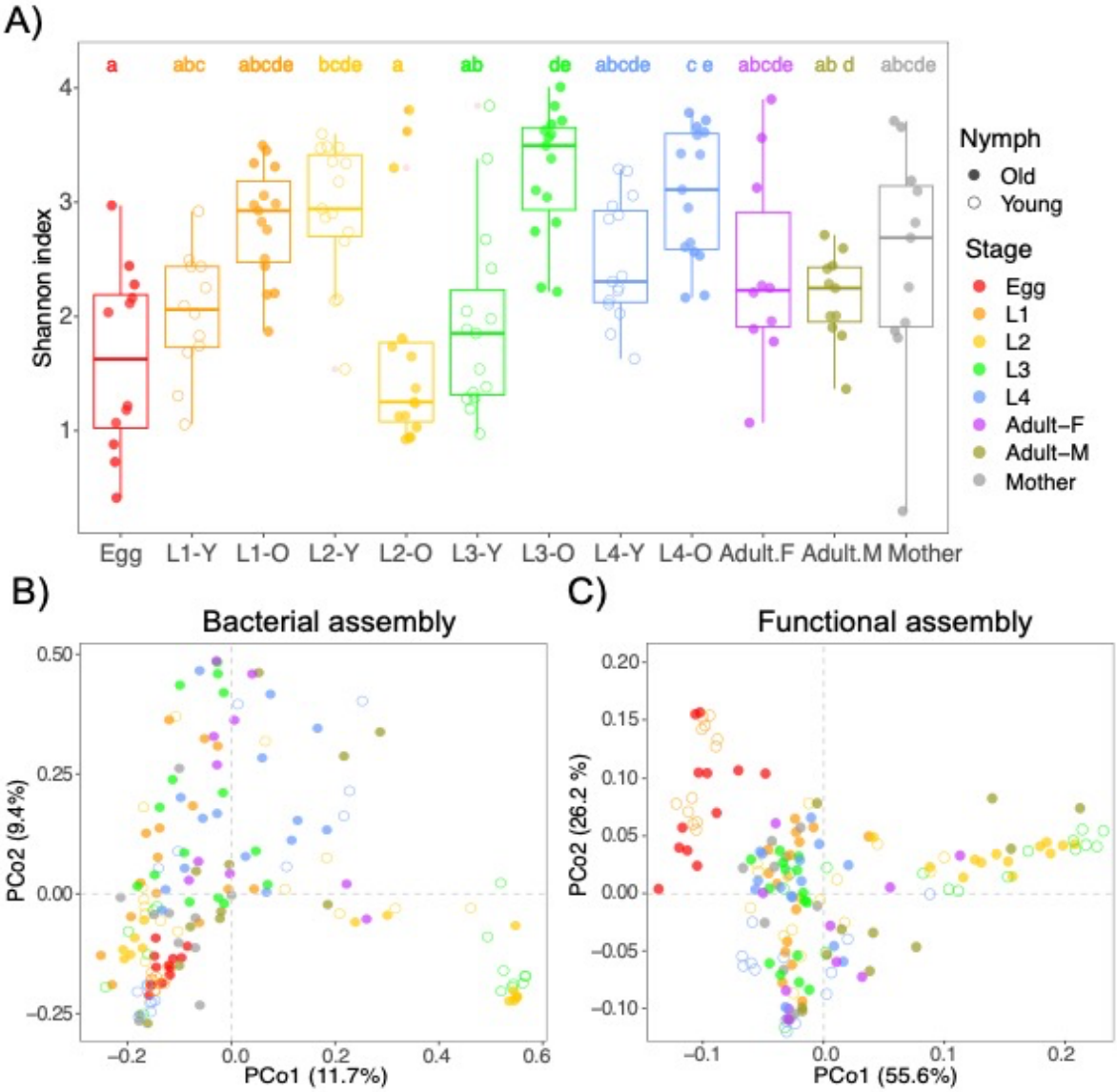


Figure 3: Microbiome variation during offspring development. The diversity is compared before (fill) and after moults (empty circles) and between each developmental stage and sex (coloured). **(A)** Shannon index comparisons for bacterial alpha diversity. Letters above the boxes indicate significant grouping contrasts ($P \leq 0.05$). Boxplots represent the median (middle bar) and the interquartile range (box) with whiskers representing the 1.5-fold the interquartile range. Dots represent the value for an individual (with red dot are outliers). **(B-C)** Principal coordinates analyses (PCoA) plots in the two first axes illustrating Bray-Curtis distances calculated between samples based on their ASVs and their KOs compositions.

395 Overall, moult was not associated with changes in offspring alpha diversity,
396 except for the final moult from 4th instar to adult males. In old nymphs, alpha diversity
397 was lower in 2nd compared to 3rd and 4th instars, whereas values were intermediate
398 in 1st instars and adults (Figure 3A). In contrast, in newly moulted nymphs, alpha
399 diversity was lower in 3rd instar compared to 2nd instar, while values were intermediate
400 in 1st and 4th instar (Figure 3A). Finally, in adult offspring, alpha diversity was similar
401 in males and females (Figure 3A, Figures S3, Supplemental File). As with alpha
402 diversity, the beta diversity of the earwig gut microbiome changed as the offspring
403 developed (PERMANOVA performed on all beta diversity metrics, Stage effect 0.074
404 < R^2 < 0.103, all P = 0.001) (Table 1).

405

406 **Table 1:** Effect of developmental stage (from eggs to adults) on microbiome alpha and
407 beta diversity at the bacterial (white) and functional (grey) levels. Significant p-values
408 (P <.005) are indicated in bold.

	Alpha diversity				**Beta diversity**			
	Index	**R^2**	**Chi^2**	**P**	**Distance**	**R^2**	**F**	**P**
Bacteria (ASVs)	Obs. richness	.436	87.23	**<.001**	Jaccard	.074	1.94	**<.001**
	Shannon	.335	59.00	**<.001**	Bray-Curtis	.082	2.50	**<.001**
	Faith	.398	71.13	**<.001**	Un. Unifrac	.065	2.73	**<.001**
	Allen	.335	78.57	**<.001**	W. Unifrac	.103	4.82	**<.001**
Functions (KOs)	-	-	-	-	Jaccard	.092	3.42	**<.001**
	-	-	-	-	Bray-Curtis	.089	3.54	**<.001**

409

410 Here, however, the beta diversity of each collection time was different from all
411 other collection times for at least one metric in 61 of the 65 pairwise comparisons (P <
412 0.05). Two major microbial turnovers occurred after hatching and during the passage

413 from the 2nd to the 3rd instar nymphs. The PCoA performed on taxonomical distance
414 matrices (*i.e.,* Bray-Curtis and Jaccard) showed a marked separation on the first axis
415 with both microbial assemblies of old 2nd instar nymphs (L2-O) and the young 3rd instar
416 nymphs (L3-Y) on one side, and all the microbial assemblies associated with the other
417 stages on the other side (Figure 3B, Figure S5A). The PCoA based on Unifrac
418 distances shows an additional separation with eggs and newly hatched nymphs (L1-
419 Y) on one side and the other stages on the other side (Figure S5B-C) showing a strong
420 phylogenetic turnover. The only four comparisons that did not present any beta
421 diversity difference were before and after the three first moults, i.e. between eggs and
422 newly hatched nymphs (L1-Y), between old 1st instar nymphs (L1-O) and young 2nd
423 instar nymphs (L2-Y), and between old 2nd instar nymphs (L2-O) and young 3rd instar
424 nymphs (L3-Y), as well as the comparison between adult males and females
425 (Supplemental File).

426

427 **Stage-specific microbes in the offspring microbiome**

428 A total of 231 ASVs that belonged to 62 bacterial genera were stage-specific (Figure
429 4). The hierarchical clustering analysis based on the Z-score of these discriminants
430 showed a structure that does not strictly follow the chronology of the developmental
431 stages of the offspring, but rather clustered individuals at the end of one instar to
432 individuals at the beginning of the following instar. In particular, they grouped the eggs
433 with newly hatched nymphs (L1-Y), the old 1st (L1-O) with the young 2nd instar nymphs
434 (L2-Y), and the old 2nd instar (L2-O) with the young 3rd (L3-Y), and the old 4th instar
435 nymphs (L4-O) with adults (Figure 4).

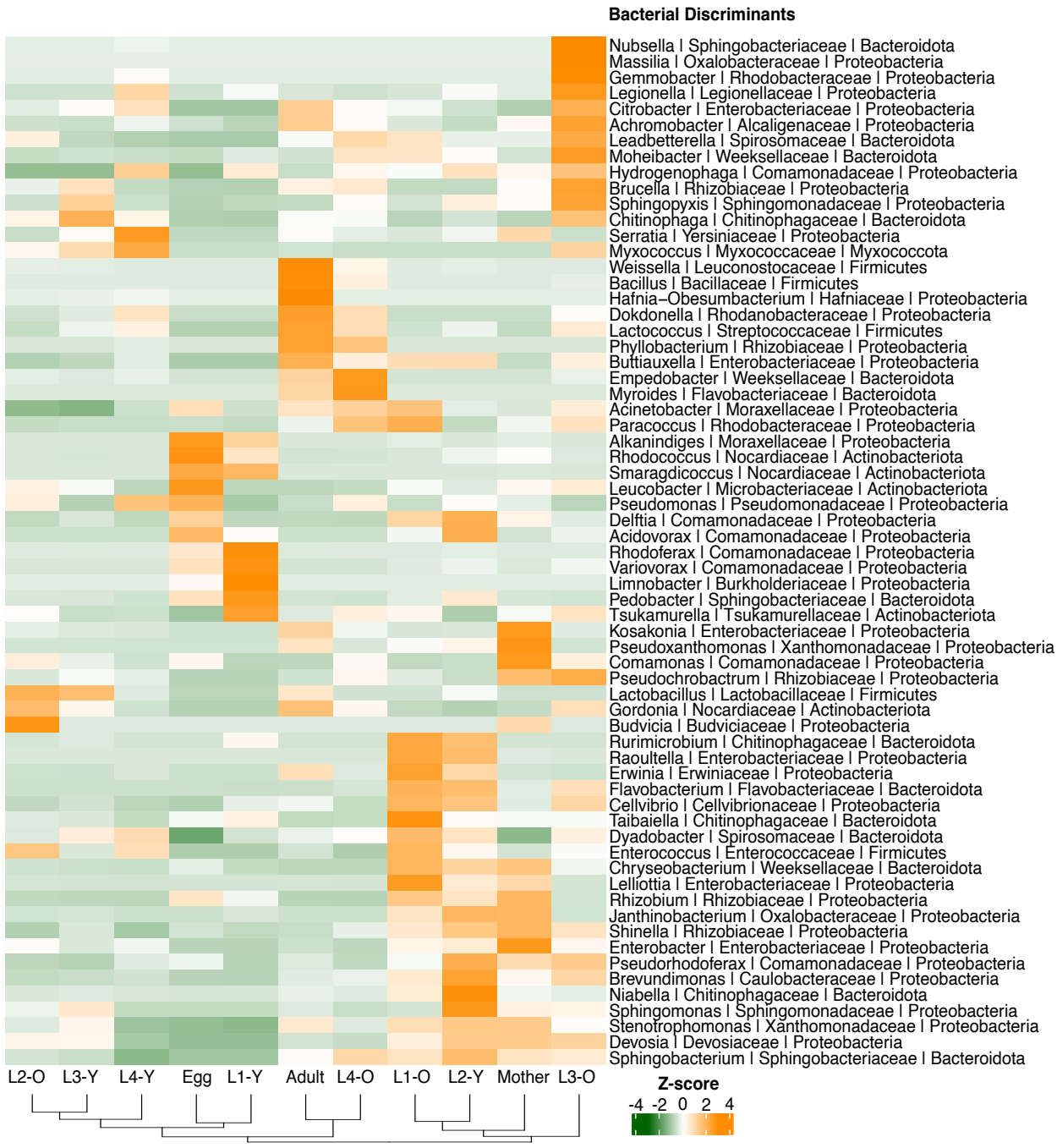


Figure 4: Heatmaps of hierarchical clustering based on the 62 genera indicators of developmental stages in the European earwig. Raw represent the Z-score associated to the bacterial genus in each sampling stage.

441 **Short- and long-term effects of maternal care on offspring microbiome**

442 The presence of the mother after hatching increased the alpha-diversity of their
443 offspring microbiome at both 1st instar and adult stages, but only when the phylogeny
444 was taken into account (Figure 5A; Faith: X^2 = 9.73, P = 0.002 and Hill1 : X^2 = 3.97, P
445 = 0.046). By contrast, it affected neither richness nor entropy (Observed richness: X^2
446 = 1.86, P = 0.172 and Shannon: X^2 = 1.21, P = 0.271) (Figure S6). In terms of beta-
447 diversity, maternal presence also affected the structure of the bacterial communities
448 (Bray-Curtis: R^2 = 0.028, P = 0.001) (Figure 5B, C; Figure S7; Table 1). Note that the
449 PERMANOVA applied to Unweighted Unifrac distances calculated on bacterial
450 assemblies might be affected by a non-homogeneous dispersion of the data as
451 individuals that lived with their mother appeared to be more dispersed (*betadisper*, F
452 = 7.7, P = 0.008). However, the ordination in Figure S7 supports a clustering, for both
453 N1 and adult stages, between the samples that lived with their mother or not.

454 The presence of a mother and the developmental stage were discriminated by
455 19 ASVs belonging to 12 genera in the microbiomes of the offspring. Among these
456 genera, *Rurimicrobium*, *Rhodoferax* and *Raoutella* were strongly discriminant in
457 individuals that had a mother during early life (Log2Fold change > |20|) while no
458 sequence belonging to these genera was present in the orphaned nymphs (Figure 5D).

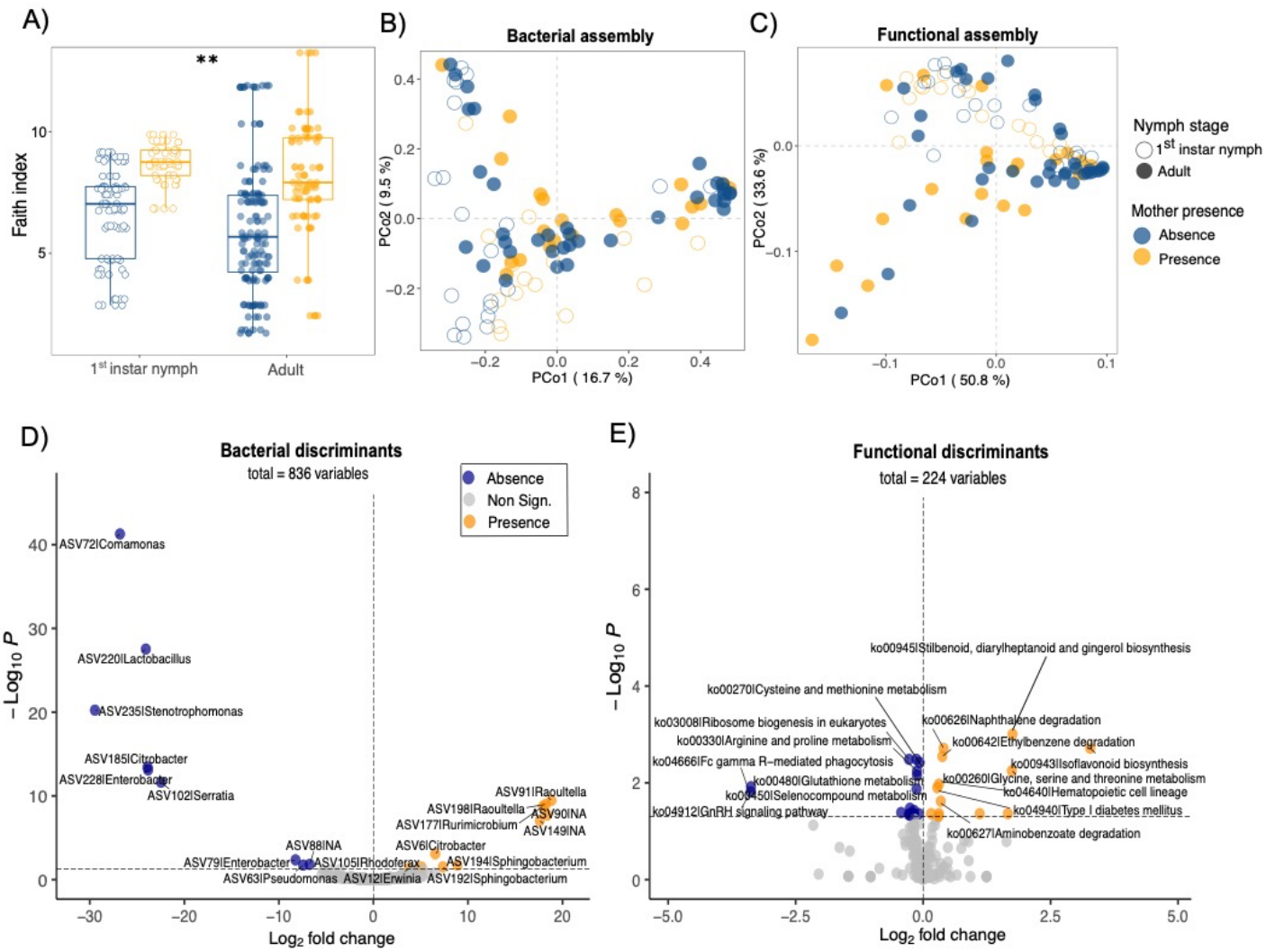


Figure 5: Effect of maternal presence (orange) or absence (blue) on the microbiome of 1st instar nymphs (empty circles) and adults (fill circles). (A) Alpha diversity is represented with the phylogenetic diversity index of Faith. **(B, C)** Beta diversity is showed through a two-dimensional PCoA according to the Bray-Curtis distances between microbiome sample pairs for bacterial and functional assemblies. (**C, D**) Volcano plots representing the 19 ASVs and the 33 KEGG pathways discriminating the absence (blue) or presence (orange) of mothers during family life. Boxplots represent the median (middle bar) and the interquartile range (box) with whiskers representing the 1.5-fold the interquartile range. P 0.001 < ** ≤ 0.01.

469 **Predictions of potential functions of the different microbiomes**

470 In terms of function, the 915 ASVs of the global core microbiome were predicted
471 to be associated with 4 952 KOs involved in 226 different KEGG pathways. Most of
472 these pathways are related to metabolic routes such as lipid, carbohydrate or
473 xenobiotics biodegradation and biosynthesis of secondary metabolites (Supplemental
474 File). These functional predictions changed throughout the development of the host
475 (Stage effect $0.089 < R^2 < 0.092$, all $P = 0.001$) (Table 1). They followed the same
476 patterns of microbial assemblies, with a clear distinction between the eggs and newly
477 hatched nymphs, the old 2nd and young 3rd instar nymphs and the rest of all stages
478 (Figure 3C, Figure S8; see Pairwise results in Supplemental File). Among the 226
479 different KOs, 212 were stage-specific (Figure 6). As for bacteria, the hierarchical
480 clustering of these discriminants showed a structure that does not strictly follow the
481 chronological order of the developmental stages of the offspring (Figure 6).

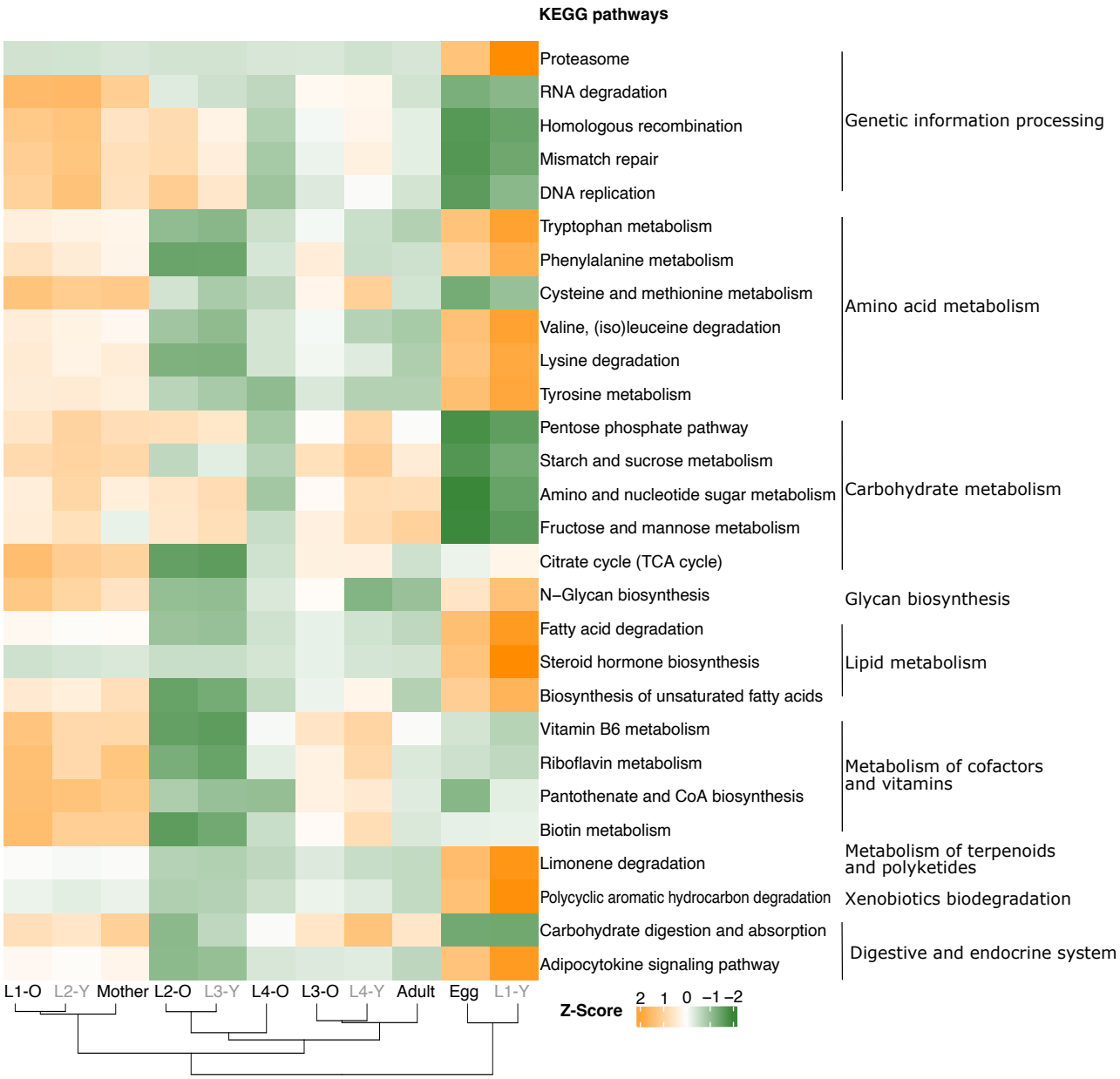


Figure 6: Heatmaps of hierarchical clustering based on the KEGG pathways indicators of developmental stages in the European earwig. Raw represent the Z-score associated to the pathway name in each sampling stage (young-nymphs are coloured in grey).

488 Maternal presence affected the predicted functional structure of the microbiome of
489 offspring (Mother presence 0.035 < R^2 < 0.040, all P < 0.009) (Table 2). This presence
490 discriminated 12 of the 226 KEGG pathways, but only three had a Log2Fold change >
491 |2.5|. These pathways included the Fc gamma R-mediated phagocytosis and GnRH
492 signalling pathway in orphans and the isoflavonoid biosynthesis in offspring that had
493 lived with their mother (Figure 5E).

494

495 **Table 2:** Effect of access to (A) maternal care during family life and (B) offspring age
496 on microbiome alpha and beta diversity at bacterial (white) and functional (grey) levels.
497 Significant p-values (P <.005) are indicated in bold.

		Alpha diversity			**Beta diversity**			
	Index	R^2	Chi^2	P	Distance	R^2	F	P
(A) Maternal care								
Bacteria (ASVs)	Obs. richness	.134	1.86	.172	Jaccard	.023	2.50	**<.001**
	Shannon	.087	.980	.323	Bray-Curtis	.028	3.28	**<.001**
	Faith	.234	8.15	**.004**	Un. Unifrac	.074	9.79	**<.001**
	Allen	.095	2.80	.093	W. Unifrac	.037	5.13	**.003**
Functions (KOs)	-	-	-	-	Jaccard	.035	4.13	**.009**
	-	-	-	-	Bray-Curtis	.040	4.82	**<.001**
(B) Nymphs vs Adults								
Bacteria (ASVs)	Obs. richness	.134	6.31	**.012**	Jaccard	.052	5.68	**<.001**
	Shannon	.137	6.05	**.014**	Bray-Curtis	.077	9.07	**<.001**
	Faith	.209	4.15	**.042**	Un. Unifrac	.108	14.16	**<.001**
	Allen	.181	12.52	**<.001**	W. Unifrac	.178	24.82	**<.001**
Functions (KOs)	-	-	-	-	Jaccard	.083	9.72	**<.001**
	-	-	-	-	Bray-Curtis	.084	10.06	**<.001**

498

499 ## Discussion

500 The gradual and subtle changes that hemimetabolous juveniles undergo during
501 moulting are often thought to limit profound changes in their microbiota during
502 development (Carrasco and Pérez-Cobas, 2014; Manthey *et al.*, 2022). Our data show
503 that this assumption is not always true, as these changes occur in the European
504 earwig. Using 16S rRNA metabarcoding on 218 samples from egg to adult stages, we
505 found that the microbiome of earwig offspring shows substantial variation throughout
506 their development both in terms of beta diversity (thereafter called "structure") and
507 alpha diversity (thereafter called "diversity"). Interestingly, these changes did not occur
508 during moulting, but rather between the beginning and end of certain developmental
509 stages. In addition, we found that maternal care partly shapes the microbiome of
510 offspring, even if this behaviour is facultative in the European earwig. Access to
511 maternal care during the first few weeks after hatching affected not only the
512 microbiome of first instar nymphs during family life, but also that of the resulting adults
513 two months after family life has ended.

514

515 **Offspring microbiome varies during development**

516 Our data first reveal that the microbiome diversity (alpha) and structure (beta) of earwig
517 changed during offspring development. This is striking for two main reasons. First,
518 these changes were not linear and only occurred at certain stages of offspring
519 development, which contrasts with the general pattern where microbiome diversity
520 gradually increases with natural growth in offspring body size (Sherrill-Mix *et al.*, 2018).
521 Second, some of these changes occurred in the second and third nymphal instars,
522 even though all instars were fed the same diet and developed in the same laboratory
523 conditions throughout their development.

524 The first marked change in microbiome structure occurred shortly after the
525 nymphs hatched. We showed that both eggs and newly hatched nymphs had a
526 microbiome structure different from that of all other stages. From the egg stage to the
527 freshly moulted 2nd instar nymphs, we observed an increase in diversity due to the
528 gradual colonization of the 1st instar nymphs by new genera, including *Erwinia,*
529 *Raoultella, Flavobacterium* and *Lactobacillus*. These new genera are present in a wide
530 range of insects, where they are often known to perform beneficial functions in
531 juveniles (Malacrinò, 2022). For instance, *Erwinia* is known to reduce the maturation
532 time of the bark beetle *Ips typographus* (Peral-Aranega *et al.*, 2023).

533 The second marked change in microbiome diversity occurred during the
534 development of the 2nd instar nymphs. We found that the diversity drops to its lowest
535 level during the transition from newly moulted to old 2nd instar nymphs and remains
536 low in the newly moulted 3rd instar nymphs (Figure 3A). This fall is reflected by the loss
537 of several genera, including *Smaragdicoccus, Rhodococcus* or *Alkanindiges* (Figure
538 4). The bacterial communities of the newly moulted 3rd instar nymphs are very different
539 from those of any other stage, because of its structural monotony where *Lactobacillus*
540 largely dominates the bacterial composition (Figures 1, 4). Such a decrease in
541 microbiome diversity has been reported in the 3rd instar larvae of the German
542 cockroach *Blattella germanica* (Carrasco and Pérez-Cobas, 2014), where it has been
543 suggested to result from the physiological state of the host at that particular stage
544 (Kirkland *et al.*, 2020). In lower termite workers, the gut flagellates are totally lost prior
545 the ecdysis, probably due to a combination of host starvation and hormonal variation
546 linked to changes in the host social status (Cleveland, 1949; Raina *et al.*, 2008; Nalepa,
547 2017). This may also be the case with earwigs, although our knowledge of the
548 physiological and hormonal peculiarities of each instar is still very limited (Meunier,

549 2024). This decrease may also result from the end of mother-offspring interactions, as
550 we removed mothers from their nymphs 14 days after hatching, which is often shortly
551 after they have reached the 2nd instar (Thesing *et al.*, 2015). This end of family life
552 inherently means that any potential form of coprophagy and trophallaxis between
553 mother and offspring (and thus potential bacterial vertical transmission) ceases, and
554 the juveniles begin to process the food source exclusively on their own. Whether
555 physiological peculiarities of 2nd instar nymphs, suppression of maternal bacterial
556 transfer and/or changes in dietary habits are the drivers of the reported changes in the
557 microbiome diversity remains to be further investigated.

558 The last marked change in microbiome diversity was an increase during the
559 development of the 3rd instar nymphs, mostly explained by the colonization of new
560 genera, such as *Nubsella, Massilia, Gemmobacter* or *Chitinophaga.* These genera are
561 common colonizers of insect guts (Da Silva Correia *et al.*, 2018; Guégan *et al.*, 2018;
562 Paddock *et al.*, 2022) but can also be found in the environment (Mayoral-Peña *et al.*,
563 2022). For example, bacteria of the genus *Nubsella* are mutualistic with certain
564 phytophagous insects and are likely to be important for the fitness of walking sticks (Lü
565 *et al.*, 2019; Li *et al.*, 2020). Interestingly, the genus *Chitinophaga* is known to be
566 involved in the degradation of chitin, a major component of the insect skeleton (Glavina
567 Del Rio *et al.*, 2010). Whether their presence is a mutualistic association with earwigs
568 aiding in cuticle digestion during cannibalism or during the reingestion of their own
569 exuviae is an open question and will be developed below.

570

571 **Moulting does not alter microbiome diversity**

572 Moulting did not induce any changes in the microbiome diversity and structure of
573 earwig offspring, except for the moults between the 3rd and 4th instar nymphs. This

574 overall lack of moulting effect is in line with studies carried out in other hemimetabolous
575 insects showing that the gut microbiome remains stable during development (Manthey
576 *et al.*, 2022). For earwigs, we propose several possible explanations. First, nymphs
577 could possess a microbial reservoir that is preserved during moulting. Such a reservoir
578 is present, for example, in the bean bug *Riptortus clavatus* to maintain their
579 *Burkholderia* symbionts during moult (Kikuchi and Yumoto, 2013). However, the
580 presence of such a reservoir has never been documented in earwigs. Another
581 possibility is that earwig nymphs re-inoculate themselves with their own bacteria by
582 eating their shed cuticle after moulting, as reported in cockroaches (Mira, 2000). Even
583 if earwig nymphs are regularly observed eating their shed cuticle during laboratory
584 rearing (J Meunier, pers. obs), the role of this behaviour to inoculate a lost microbiome
585 has never been investigated in this species. Finally, earwig nymphs may be able to re-
586 inoculate themselves through their frequent (in)direct contact with their siblings by
587 means of coprophagy and trophallaxis (Falk *et al.*, 2014; Kramer and Meunier, 2016).
588 These social acquisitions allow the persistence and the (re)acquisition of lost bacteria
589 in numerous arthropods, such as termites (Raina *et al.*, 2008; Michaud *et al.*, 2020),
590 cockroaches (Nalepa, 2020), and spiders (Rose *et al.*, 2023). Overall, these data call
591 for future experiments to disentangle which parameter explains the resistance of the
592 earwig microbiome to moulting, and to understand why they are no longer efficient for
593 the moult between the 3rd and 4th instar nymphs.
594

595 **Bacterial functions could play a role throughout juvenile development**

596 The reported changes in the structure of the offspring microbiome during development
597 are associated with changes in the predicted potential functions of their bacteria that
598 could be beneficial to the host. For instance, many of the bacteria overrepresented in

599 eggs and newly hatched nymphs are known to produce and accumulate lipids such as
600 *Rhodococcus, Delftia* and *Pedobacter* (Alvarez *et al.*, 1997; Liu *et al.*, 2016; Franks *et*
601 *al.*, 2021). As completing the transition from eggs to nymphs is a highly energetic
602 process, our data suggest that earwig embryos may not only obtain this energy from
603 egg lipid reserves, but also from these bacteria (Ziegler and Vanantwerpen, 2006;
604 Diether and Willing, 2019). Moreover, this would be consistent with the predictions of
605 PICRUST2, which show a strong positive correlation in lipogenesis processes such as
606 adipocytokine signalling pathway and unsaturated fatty acid biosynthesis (Figure 6).
607 The bacterial loss observed in the third nymph instar was also found in terms of
608 predicted functions, as all pathways except cell developmental pathways, amino acids
609 and sugar metabolism were underrepresented in this developmental stage compared
610 to the subsequent ones. Finally, the acquisition of new genera such as *Nubsella,*
611 *Massilia, Gemmobacter* or *Chitinophaga* during the development of the third nymphal
612 instar came with new predicted functions linked to energy uptake, amino acids and
613 vitamins B biosynthesis, which act as a coenzyme in numerous pathways involved in
614 the fatty acid synthesis, glucogenesis or amino acids synthesis. These vitamins are
615 essential during insect development, but they cannot be synthetized by animals
616 themselves and are often acquired through alimentation or provided by the microbiota
617 (Douglas, 2017; Kinjo *et al.*, 2022). Although these functions may provide insights into
618 our general understanding of the driver of microbiome changes during offspring
619 development, they must be considered with caution as the validity of the approach is
620 debatable due to the short length of the amplicons and the lack of genome reference
621 concerning insect-associated microbial communities (Djemiel *et al.*, 2022). Future
622 studies are thus needed to confirm these potential functions in earwigs, for example
623 using transcriptomic analyses.

624

625 **Maternal care shapes the microbiomes structure of offspring**

626 In animals, maternal care is often considered to be an important mediator of microbial
627 transmission from parents to offspring (Bright and Bulgheresi, 2010; Sachs *et al.*, 2011;
628 Hosokawa and Fukatsu, 2020). Our data suggests that the European earwig is no
629 exception. Access to maternal care not only shapes the microbiome structure,
630 phylogenetic diversity and functions of 1st instar nymphs, but also those of the resulting
631 adults, even though none of these adults had any contact with their mothers in the
632 previous two months. We found that the microbiome of nymphs with maternal care
633 contained a higher diversity of phylogenetically distant bacteria compared to their
634 orphaned counterparts. Not surprisingly, the bacteria found in these nymphs were also
635 found in the microbiome of their mothers (including *Raoultella*, *Rurimicrobium* and
636 *Sphingobacterium*). This is likely due to direct or indirect maternal transmission during
637 post-hatching family life. However, even if maternal transmission contributes to the
638 shape of the microbiome, the non-significant effect of maternal care on alpha diversity
639 in terms of taxonomic richness, Shannon entropy or Allen index, combined with the
640 small, albeit significant effect on microbial structure, suggests that maternal presence
641 is not the main route of bacterial acquisition in this species. Indeed, we also found an
642 overabundance of some ASVs in the orphan offspring, such as sequences related to
643 *Comamonas*, *Lactobacillus* or *Serratia*. Since these bacteria are often generalists,
644 associated with laboratory rearing conditions (Malacrinò, 2022), and common in the
645 mothers tested, they are likely to come from the rearing environment. However, their
646 overabundance in orphaned nymphs suggests that they were outcompeted by
647 maternally transmitted bacteria in non-orphaned nymphs. In addition to these
648 differences, we found one major discriminant (potential) function in the tended nymphs,

649 related to isoflavonoid biosynthesis, and two in the orphan nymphs, related to the
650 endocrine and immune systems. These predicted functions are consistent with
651 previous phenotypic studies showing that orphaned nymphs develop faster to adults,
652 produce larger adults with longer male appendages, but contradict with other studies
653 showing that orphaning has limited long-term effects on the basal immunity of the
654 nymphs and resulting adults (Meunier and Kölliker, 2012; Thesing *et al.*, 2015;
655 Vogelweith *et al.*, 2017; Körner *et al.*, 2020). Here again, these predicted functions
656 need to be taken with caution. Whether all or some of these offspring phenotypes are
657 indeed due to maternally derived bacteria remains to be further explored, *e.g.* with the
658 use of gnotobiotic lineages.
659

660 ## Conclusion

661 Overall, we showed that the European earwig microbiome changes multiple times
662 during offspring development. Interestingly, these changes were independent of
663 moulting. The fact that moulting did not induce any purge or shift in the bacterial
664 communities of the nymphs calls for future studies to test whether this is due to the
665 presence of a bacterial reservoir, moult consumption and/or social interactions with
666 siblings. Our data suggest that the predicted functions of some components of these
667 microbiomes are relevant to the developmental stage at which they occur, such as
668 lipogenesis or steroid synthesis in early stages, and nutrient and vitamin synthesis in
669 late stages. However, future studies are required to confirm the functional role of the
670 microbiome changes in this species. Finally, we showed that maternal care is an
671 important short- and long-term determinant of the offspring microbiome. Given that
672 earwig nymphs do not require maternal care to develop and survive (Kölliker, 2007;
673 Thesing *et al.*, 2015; Kramer and Meunier, 2016) and that nymph can also develop in

absence of any social interactions (Van Meyel and Meunier, 2022), our results call for future studies to investigate the role of these socially-acquired bacteria (and other potential members of their microbiota such as fungi, viruses and other microorganisms) in the biology of the European earwig and, more generally, in the early evolution and maintenance of facultative family life in insects (Archie and Tung, 2015; Körner *et al.*, 2023).

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Author Contributions

All authors contributed to the study conception. The manuscript was written by M-C Cheutin and J. Meunier; Analyses were carried by M-C Cheutin and J. Meunier; Molecular analyses, sampling and animal rearing were performed by M-C Cheutin and M. Boucicot. All authors read and approved the final version of the manuscript.

Data Availability

The data set and scripts are available on Zenodo with the DOI https://zenodo.org/doi/10.5281/zenodo.10776543. Libraries for each sample are deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession no. PRJNA1066258.

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Conflict of interest

We declare that our work does not have any conflict of interest.

Ethics approval statements

Our investigation complies with the current European Directive 2010/63/EU that does not acquire ethical approval on invertebrates. All animals were handled with care until necessary sacrifices.

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