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# Ecology of vertical tumor transmission in the freshwater cnidarian *Hydra oligactis*

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**Transmissible tumors are increasingly regarded as a new form of parasitic life, but relatively little is known about the ecology and evolution of their interactions with their host. In this work, we provide new insights into transmission dynamics of vertically transmitted tumors in the freshwater cnidarian *Hydra oligactis*. First, we found tumoral hydra to be infectious at any age, regardless of whether they were in their asymptomatic or symptomatic phases, with the bacteriome composition remaining constant during both phases. Interestingly, tumor transmission increased with the number of tentacles, particularly for hydras with supernumerary tentacles. Additionally, tumors developed earlier in the offspring from parents with more advanced tumors. Furthermore, despite being direct descendants of tumoral polyps, some hydras never developed tumoral phenotype. The latter exhibited a distinct bacteriome composition, reduced lifespan and a lower tentacle number increase over time. Interestingly, the tumor phenotype expression in these hydras appears to be able to skip generations, as transmission occurred at any age from parents to offspring. We discuss these results in the context of current knowledge on the evolutionary ecology of host-transmissible tumor interactions as well as parasite-host interactions and suggest avenues for further research.**

**Keywords** Hydra, Host-tumor interactions, Vertical transmission, Tumor ecology, Life-history traits, Bacteriome

The emergence of multicellularity towards the end of the Precambrian era was dependent on the evolution of cooperative interactions among cells. However, a new challenge arose with the advent of mutant "cheater" cells that act selfishly to increase their own fitness at a cost to that of the multicellular organism[1]. These cells violate the rules that govern proper functioning in metazoans (e.g., the inhibition of proliferation, controlled cell death, allocation of resources, division of labor, and the creation and maintenance of the extracellular environment), which gives them a selective advantage, at least transient, over healthy cells[2,3]. Failure to eliminate these "cheater" cells through the many regulatory mechanisms that evolved in animals (e.g.,[4]) can result in the formation of abnormal tissue growth (resulting in a tumor or neoplasm), potentially having devastating consequences for the organism's fitness. This includes not only death caused by the pathology itself, but also fitness reduction due to compromised competitive abilities, heightened susceptibility to predation and pathogens, and a diminished capacity for dispersal[5–7]. While tumors, both benign and malignant, are ubiquitous across multicellular life[3,8], our understanding of the ecology and evolution of host-tumor interactions in the wild remains limited[9].

In the vast majority of cases, tumors die with their host. However, some forms have acquired the ability to transmit between individuals, sometimes even across different species (see[10] for review). Currently, several naturally-occurring transmissible tumor types have been documented: Canine Transmissible Venereal Tumor (CTVT) in dogs[11,12], Tasmanian Devil Facial Tumor Diseases (DFTD1 and DFTD2) in Tasmanian devils[13,14], Bivalve Transmissible Neoplasia (BTN) in different bivalve species[15] and transmissible tumors in freshwater cnidarians from the genus *Hydra*[16]. For the tumors CTVT, DFTD and BTN, transmission is horizontal, meaning that it occurs between individuals which are not necessarily related, and/or even between species (e.g., BTN). On the other hand, transmissible tumors in *Hydra* represent a rare case of tumors transmitted vertically, i.e., between parent and offspring (see below). The potential for tumor cells to be transmitted between hosts suggests

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that they undergo similar evolutionary dynamics as infectious agents, thus classifying them as a "new species of parasites"[17–19]. The intersection of oncology and parasitology introduces an innovative research field that challenges conventional notions of cancer by expanding beyond the idea of uncontrolled cell growth within an individual, offering potential implications for both human health and wildlife conservation[10,20]. However, this field remains largely unexplored, mainly due to the apparent scarcity of biological models (see[21,22]) and/or the difficulty of studying them in nature, which limits our understanding of the evolutionary ecology of host-transmissible tumor interactions[23]. For instance, while it is generally accepted that symptomatic individuals with tumors can transmit tumor cells to others, the extent to which asymptomatic individuals (i.e., bearing early-stage tumors) can also transmit tumor cells is poorly documented. Similarly, among symptomatic individuals, it is unknown if a correlation exists between the extent of symptom manifestations in tumor-bearing individuals and the tumor cell transmission rate. We believe that the vertically transmissible tumor system in *Hydra*, especially because of its ease of maintenance in the laboratory[16,24], is an appropriate model for answering these types of questions and for providing new insights into the ecology and evolution of host-tumor interactions.

*Hydra oligactis* is capable of both sexual and asexual reproduction[25]. In this species, notably the Saint Petersburg tumorous hydra line (TL), maintained asexually in culture for over 15 years, tumor cells and the specific bacteriome associated with them are transmitted (i.e., from parent to offspring) during asexual reproduction via budding[16,26]. The newly produced TL buds (i.e., detached individuals), under commonly used rearing conditions (i.e., fed three to five times a week), develop a tumorous phenotype within approximately four weeks[26–28]. Interestingly, this transmission is not consistent, resulting in the production of rare healthy descendants from tumoral line hydras (HDTL), showcasing distinct bacteriome compositions[16,26,27]. Also, tumorigenesis alters the external phenotype of hydras at various levels. While healthy hydras are thin and typically have 6 to 7 tentacles, symptomatic hydras (SH), exhibit tumefactions visible to the naked eye and can develop between eight and up to twenty tentacles[5,26]. These phenotypic alterations, interestingly, impact ecological dynamics: SH have been demonstrated to capture more prey than healthy hydras and, conversely, are more susceptible to predation[5]. Additionally, before becoming symptomatic, asymptomatic hydras (AH) show modifications in their life-history traits: similar to what is sometimes observed in hosts infected by lethal or castrating parasites[29,30], AH maximize their effort for early asexual reproduction before tumors significantly impact their budding capacity and survival rate[27].

In order to enhance our understanding of the evolutionary ecology of vertically transmissible tumors, we have sought to address the following questions using the hydra model: (I) To what extent asymptomatic polyps (but capable of developing tumors later) present a risk of tumor transmission to their offspring? (II) Once a polyp is symptomatic, is there a correlation between the age of tumor onset, the degree of symptomatology exhibited by a polyp, and the time when tumors appear in its descendant buds? (III) Do the rare HDTL have particular life-history trait characteristics compared to those that have been infected, and do they infect their descendant buds? (IV) What are the dynamics of the bacteriome before and after tumor development? (V) Is the presence of supernumerary tentacles associated with a distinct bacteriome composition?

## Materials and methods

### Animals and culture conditions

Experiments were conducted using TL, derived from the clonal lineage of Domazet-Lošo et al.[16]. This strain was maintained under a feeding regime of three time per week, following standard rearing protocols described in Boutry et al.[27]. For the experiment (refer to the experimental protocol below), hydras displaying visible tumors were isolated from cultures and individually placed in wells of cell culture plates (12-well plates, 1.5 mL/well, Thermo Scientific). Descendants from these hydras and their subsequent buds were also isolated under the same conditions to be monitored throughout the experiment.

### Experimental protocol

The graphical summary of the general experimental design is illustrated in Fig. 1A. In order to study the tumor transmission rate to the offspring in both pre- and post-tumor appearance phases (asymptomatic and symptomatic phases), we individually isolated 18 tumorous *H. oligactis* in an advanced symptomatic phase (meaning visible tumors on the main hydra body, and the presence of supernumerary tentacles, see Fig. 1B for differentiation between symptomatic and asymptomatic hydras). These individuals constituted our first generation and were referred to as grandparents. The first two buds of the grandparents (F0) were individually isolated, and their emergence dates (further referred to as birth or birth dates), as well as the number of tentacles at birth (t0), were recorded. This resulted in 36 asymptomatic individuals (F1) of the second generation, referred to as parents. Each week, for 9 weeks, we recorded the number of tentacles, the date of the first budding, lifespan, and the health status (symptomatic or not) of the F1 parents. The nine week interval was chosen based on the known development of tumors in this specific hydra lineage, typically observed after approximately 4 weeks[26–28]. In order to capture both the asymptomatic and symptomatic phases, the duration of the experiment was determined by allocating 4 weeks to each phase, with an additional week to account for potential individual variations. Additionally, each week, the first two F2 buds of the F1 parents were isolated on their date of birth. We chose to isolate two buds per week to improve experimental reliability and accommodate for the possible loss of a F2 bud from a F1 parent. Any surplus buds produced within the same week of the F1 parent's life were not considered and discarded. This protocol aimed to obtain a maximum of 72 buds per week of the F1 parent's life. Each week, for 6 weeks, we monitored the F2 buds for the number of tentacles, lifespan, and their symptomatic/asymptomatic status. We chose a period of 6 weeks, because we assumed that tumor development, resulting from the transmission of tumor cells between the F1 parents and the F2 buds would have had sufficient time to progress to the point of being visually detectable (based on[16,27,28]).

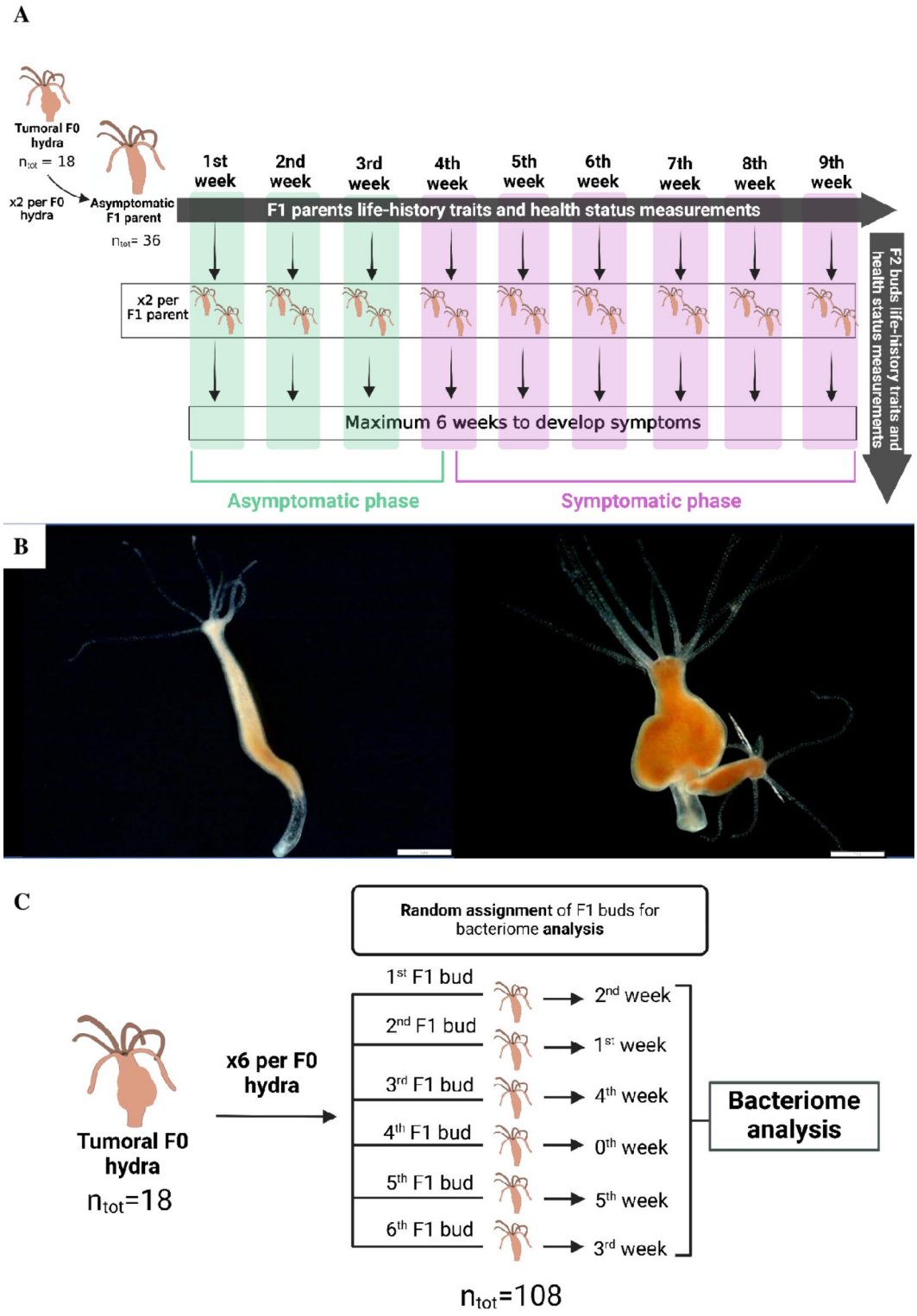


**Fig. 1**. (**A**) Graphical summary of general experimental design. (**B**) Phenotype of asymptomatic and tumoral hydras from the tumoral clonal strains of St. Petersburg. (left panel) Asymptomatic hydra: the body is thin, without supernumerary tentacles (i.e., fewer than 8). (right panel) Tumoral hydra: tumefactions thicken the body, with supernumerary tentacles (i.e., 8 or more). Scale bar: 1 mm. (**C**) Graphical summary of bacteriome experimental design.

The experimental protocol for bacteriome analysis is summarized in Fig. 1C. The same 18 tumoral F0 grandparents used in the transmission experiment were used. For each of these F0 grandparents, we isolated 6 F1 buds, assigning them ranks from 1 to 6 based on their emergence birth order. Before isolating the F1 buds, we randomly assigned them an order of freezing, corresponding to the week they were frozen, to arrest their growth (and potentially that of their bacteriome). Thus, at t0 (birth) and every week for 5 weeks, we randomly froze one F1 bud out of the 6 for each F0 grandparent, regardless of their birth date. This randomization was performed to ensure that any unaccounted variables or factors influencing the bacteriome composition were evenly distributed across the various frozen batches. After 5 weeks, our goal was to accumulate 108 frozen F1 polyps ready for bacteriome analysis. However, one out of the six F0 grandparents produced only one F1 polyp. Consequently, we supplemented our dataset with an additional F0 grandparent. Some other F0 grandparents did not produce their full set of 6 F1 buds, and, among them, some died before the end of the experiment, resulting in 104 hydras in total. Metabarcoding of 104 F1 hydras was performed through a 16S rDNA, following the protocol adapted from Boutry et al.[31]. This included DNA extraction with the DNeasy Blood & Tissue kit (QIAgen GmbH, Hilden, Germany), amplification of the V4 region of the ribosomal 16S gene, barcoding and Illumina sequencing at the GenSeq platform (Genseq, Montpellier University).

## Data analysis

Data processing and statistical analyses were performed using the R software (version 4.2.2)[32] within the RStudio integrated development environment[33], and various packages (see the complete list in the electronic supplementary material).

Generalized linear mixed-effects models (GLMM) constructed with the glmmTMB package[34] were used for the transmission dynamics in TL, as well as the transmission dynamics and life-history changes in HDTL sections. We followed a model selection procedure described in Zuur et al.[35]: first, we determined the random effect structure using the full model and Restricted Maximum Likelihood (REML); second, we determined the fixed effect structure using Maximum Likelihood (ML); and finally, we refitted the best model using REML. To identify the most parsimonious model, the selection of random and fixed effects to include in the analyses was determined through model comparison using the corrected Akaike Information Criterion (AICc) and the associated AICc weight from the MuMIn package[36]. The fit of the data to the model was evaluated using the DHARMa package[37] through examination of residual distribution (Kolmogorov-Smirnov test, over-dispersion and outlier tests).

For bacteriome analysis, we used the FROGS pipeline[38] on the GenoToul genomic platform in the Galaxy interface for data processing. Sequences clustering into OTUs (Operational Taxonomic Units) were performed using the swarm clustering method[39]. Rare OTUs were filtered out by retaining only those with a minimum abundance of 0.005% of the total sequences, as recommended by Bokulich et al.[40]. OTUs were taxonomically assigned using 16S SILVA 138.1 reference database. Taxonomic assignments were filtered using BLAST with a minimum identity of 0.8 and a minimum coverage of 0.8. To standardize OTU abundances, rarefaction-based normalization[41] was applied to our data, resulting in a consistent read count across samples. Abundance plots of OTUs were created using the Phyloseq object[42] generated in the FROGS pipeline. The same Phyloseq object was also used for both alpha and beta diversity analyses.

### *Transmission dynamics in TL*

We aimed to understand the dynamics of tumor transmission between F1 TL parents (including both AH and SH) and their F2 buds, focusing on two key objectives: (1) the probability of tumor transmission between F1 TL parents and the F2 buds, and (2) the correlation between the age of tumor onset in F1 TL parents, their degree of symptomatology, and the age of tumor onset in their F2 offspring. During the experiment, we obtained 324 F2 buds from the 29 F1 TL parents. In total, 13 F2 buds that died before 3 weeks (the average time of tumor development in our experiment) without developing tumors were excluded due to uncertainty regarding their health status. Additionally, 11 buds were excluded from the analysis due to missing parental tentacle number information. To address these research objectives, we modeled two response variables: the health status of F2 buds (asymptomatic or symptomatic, binary) and the age of tumor onset in F2 buds (continuous), represented as difference in days between the date of the tumor onset and the birth date.

Several predictor variables were common in both analyses. One of the predictors was Δb, a continuous variable representing the difference between the F2 bud's birth date and the F1 parent's tumor onset date. It represents both the F1 parent's health status and the time since emergence (hereinafter referred to as age) at the birth of the F2 bud. A negative Δb value indicated that the F1 parent was asymptomatic at the F2 bud's birth, while a positive value indicated that the F1 parent was symptomatic at the F2 bud's birth. The absolute value of Δb represented either the time remaining until tumor onset or the time elapsed since tumor appearance. Other predictor variables included the number of tentacles of the F1 parent at the week of F2 bud birth (continuous), and the order of F2 bud birth (first or second, binary). The latter was included to account for deviations from the expected experimental protocol, where some F1 parents produced only one bud instead of the anticipated two, potentially affecting tumor transmission (i.e., the uneven representation of F2 buds from the same F1 parent). A summary of these deviations is provided in electronic supplementary material, Table S1. To account for the lack of independence between measurements, we included random intercepts for grandparental, parental and bud identifiers in the model, along with the nested effect of parental identifiers within grandparental identifiers and bud identifiers within parental identifiers.

To assess the probability of tumor transmission between F1 TL parents and F2 buds, we modeled the status of F2 buds (asymptomatic or symptomatic) using a binomial distribution with a logit link function. The predictor variables for this model included the parent's age at the time of F2 bud birth, as well as the previously described Δb, the number of tentacles of the parent at the week of F2 bud birth, and the order of F2 bud birth. Due to a high

correlation between Δb and the parent's age at the time of F2 bud birth (Spearman correlation coefficient = 0.92), these variables were analyzed separately to avoid collinearity-induced bias. Given their close correlation, we anticipated that the selected model to be very similar between the two variables. The statistical analysis was performed on a maximum of 297 F2 buds.

To investigate the factors influencing the age of tumor onset in F2 buds, we modeled the age of tumor onset in F2 buds using a negative binomial distribution with a log-link function. Here, negative binomial distribution with a log-link function was used since our response variable represents the number of failures (here, the number of days) before success (here the tumor onset). The predictor variables for this model included the age of tumor onset in F1 parents, as well as the previously described Δb, the order of F2 bud birth, and the number of tentacles of the parent at the week of F2 bud birth. Only F2 buds which developed tumors during the experiment were included in this analysis. Thus, the statistical analysis was performed on a maximum of 247 F2 buds.

*Transmission dynamics and life-history changes in HDTL*

The main goal of this analysis was to determine whether HDTL exhibit distinct life-history traits compared to TH and assess whether F1 HDTL parents transmit tumors to their descendant F2 buds. For all subsequent analyses, HDTL were defined as those which did not develop symptoms during the experiment and survived beyond the first 3 weeks (the average time of tumor development in our experiment), to avoid uncertainty in their classification. This represented a total of 6 individuals in the F1 parent generation and 69 individuals in the F2 bud generation. For transmission analysis, only F2 buds originating from F1 HDTL parents were considered ($n = 68$).

Transmission dynamics in HDTL The probability of transmission between the F1 HDTL parents and the F2 buds was modeled using a binomial mixed-effects model. We used the same response, explanatory, and random intercept variables as previously detailed for the analysis of tumor transmission in TH parents, with the summary of deviations for HDTL parents presented in electronic supplementary material, Table S2. However, a supplementary analysis with the Δb variable instead of the F1 parent's age at the F2 bud's birth variable was not conducted, as it was not possible to calculate the Δb variable for HDTL parents.

Life-history changes in HDTL To compare life-history traits, we analyzed differences in tentacle number, age at first reproduction, and survival between HDTL and TL individuals. Tentacle number evolution between HDTL and TL buds was modeled using a Gaussian mixed-effects model with an identity link function. The number of tentacles per week was included as the continuous response variable. We included the health status of the F2 bud (HDTL or TL, binary), the age of F2 bud (in weeks, continuous), as well as the number of tentacles of the F1 parent at the week of F2 bud birth (continuous). Here, we used a Gaussian model instead of a Poisson model because our response variable followed a normal distribution. To address the non-independence of measurements, we incorporated random intercepts for both parental and bud identifiers in the model, while also considering the nested effect of parental identifiers within bud identifiers. We used a total of 2413 observations of F2 buds for the analysis.

The age at first reproduction between F1 HDTL and TL parents was compared using a negative binomial mixed-effects model, with the Δfb as the response variable. The Δfb represents the difference (in days) between the F1 parent's date of birth and the date of appearance of its first F2 bud. We included the health status of the hydra (HDTL or TL, binary) as an explanatory variable. Here, negative binomial distribution with log link function was used since Δfb variable represents the number of failures (here, the number of days) before success (first F2 bud appearance). The lack of independence between measurements was addressed by incorporating random intercepts for parental and grandparental identifiers in the model. A total number of 35 F1 parents was used for the analysis.

Survival probability between HDTL and TL was assessed using parametric survival regression with the Weibull distribution, implemented in the survival package[43,44]. The status of the hydra was included as a binary explanatory variable (HDTL or TL). Since the durations of the experiments for F1 parent and F2 bud generations were not the same (9 weeks for F1 parent vs. 6 weeks for F2 bud), we standardized the analysis to a 6-week duration, resulting in total of 309 individuals (35 F1 parents and 274 F2 buds). As part of this standardization, we excluded 108 F2 buds produced by the F1 parents on their 7th, 8th, and 9th weeks. Here, Weibull distribution was used due to the non-constant hazard observed in the data. Kaplan-Meier survival curves were used to illustrate the comparison of survival probabilities between the two groups.

*Bacteriome*

We aimed to explore bacterial diversity across different conditions (AH, SH and HDTL), and across various ages. Specifically, we sought to examine the dynamics of the bacteriome before and after tumor development, and whether the presence of supernumerary tentacles was associated with a distinct bacteriome composition.

All hydras that did not develop tumors in weeks 0, 1, and 2 were considered as AH; individuals beyond 3 weeks (the average time of tumor development in our experiment) were considered as HDTL. We created a new binary variable representing the number of tentacles as equal to or above 8 (8+) or 7 and below (-7). To maintain taxonomic consistency, all analyses were conducted at the order level due to numerous multi-affiliations observed at the species and family levels.

Alpha diversity was measured using the Chao1, Inverse Simpson and Shannon indexes based on OTU presence and abundance. The Chao1 index provides a measure of species richness within a sample, representing the count of OTUs. Meanwhile, the Shannon and Inverse Simpson indexes serve as indicators of community diversity, encompassing both species richness and evenness in the community[45,46]. Non-parametric Kruskal-Wallis and Wilcoxon rank-sum tests, along with non-parametric Dunn post-hoc tests, were used for the

examination of overall differences and pairwise comparisons between alpha indices and variables of interest. The p-value obtained from pairwise comparisons was adjusted using a Benjamini–Hochberg false discovery rate correction[47].

Beta diversity measures were calculated using the Bray-Curtis and Jaccard indexes based on OTU presence or absence and their abundance. The Bray-Curtis index assessed dissimilarity in OTU composition and abundance between samples, while the Jaccard index focused on the OTU presence or absence[45,46]. To test for significant differences in the abundance of taxa of interest across different groups, non-parametric Kruskal-Wallis tests followed by Dunn's non-parametric post-hoc tests were employed. Overall and pairwise comparisons in differences of beta diversity were carried out using permutational multivariate analysis of variance (PERMANOVA). Same p-value adjustments as previously described for alpha diversity measures were used. Principal coordinates analysis (PCoA) were carried out based on the beta-diversity index matrix.

## Results

### Transmission dynamics in TL

The measured raw average transmission rate in TL over the whole experiment was 87% with some small non-significant week to week variations (range: 69-100%) (Fig. 2A). The age and health status of the parents had no significant effects on the transmission probability. In 78 out of 107 cases (73%), buds developed tumors during the asymptomatic phase of their parent, up to 6 weeks before their parent developed symptoms (Fig. 2B). The number of parental tentacles on the week a bud was born, and the variation between the parental hydras lines (random effect), were identified as factors explaining differences in transmission rates between the F1 parents and the F2 buds (GLMM (logit link); Odds Ratio (OR) = 2.29, SE = 0.3, $N = 297$, p-value = 0.002). Specifically, transmission rates increased depending on the number of supernumerary tentacles of the parent with raw transmission rates detailed in Fig. 2C.

The age at which a bud developed a tumor was influenced by how close its parent was to develop its own tumor, but also by variations between parental hydra lines (Fig. 3, GLMM (log-link); Incidence Rate Ratio (IRR) = 0.99, SE = 0.001, $N = 247$, p-value < 0.001). For instance, a bud detached 30 days before its parent developed a tumor would, on average, develop a tumor after 29 days. In contrast, a bud that detached 40 days after its parent developed a tumor would, on average, develop a tumor after only 16 days (Fig. 3). Thus, the longer a parental hydra has been bearing a tumor, the faster its buds developed tumors. Conversely, the age of parental tumor onset, as well as the parental tentacle number at the week of bud birth, showed no effect on the age of tumor onset in buds.

### Transmission dynamics and life-history changes in HDTL

*Frequency of HDTL appearance*

Among the F1 parent generation, 6 out of 35 remained healthy, representing a percentage of 17% in the offspring of the F0 grandparents. For the buds from the F2 generation, 69 individuals out of 379 remained asymptomatic, which translates to a percentage of 18%.

*Transmission dynamics in HDTL*

HDTL showed to be infectious to its descendant buds, averaging 72% transmission rate over the course of the experiment, with minor, non-significant week-to-week variations (range: 0-100%), including the 0% observed in week 1 coming from a single individual (Fig. 4A). The order of bud birth, as well as variation between parental hydra lines (i.e. random effect), were factors explaining differences in transmission rates between the F1 HDTL parents and their F2 buds. While significant, the effect of the bud order was characterized by a very low effect size and thus its influence on the transmission rate is minimal (GLMM (logit link); OR = 0.08, SE = 1.27, $N = 68$, p-value = 0.04). The age and tentacle number of the parent at bud birth had no significant effects on transmission probability, indicating that the tumor transmission rate in buds remains constant within a parental HDTL lineage (raw transmission rates are detailed in Fig. 4A and B).

*Life-history changes in HDTL*

When examining tentacle number changes between HDTL and TL buds, we observed a significant interaction between the health status of F2 buds and their number of tentacles. Indeed, the number of tentacles increased at a larger rate over time in TL compared to HDTL (Fig. 5A, GLMM (identity link); estimate = 0.17, SE = 0.028, $N = 2422$, p-value < 0.001). In addition, the number of tentacles in F2 buds tended to increase with age (Fig. 5A, GLMM (identity link); estimate = 0.21, SE = 0.025, $N = 2422$, p-value < 0.001). Finally, the number of tentacles of F2 buds was positively associated with the number of tentacles of their F1 parents (Fig. 5B, GLMM (identity link); estimate = 0.19, SE = 0.04, $N = 2422$, p-value < 0.001). On average, for each additional tentacle in the F1 parent, the F2 buds tend to show an increase of 0.19 tentacles.

In terms of age at first reproduction, no significant difference was observed between HDTL and TL parents (Fig. 5C, GLMM (log link); the best-fitted model does not include the effect of any parameter, $N = 35$). The average age at first budding rate estimated by the model was approximately 11 days.

Survival analysis revealed that being HDTL significantly influenced the risk of death (Weibull survival regression: estimate = -0.41, scale = 0.23, SE = 0.09, $N = 309$, p-value < 0.001). HDTL had a 5.82 times higher risk of dying during the experiment compared to TL (Fig. 5D, Hazard Ratio = 5.82, 95% CI: 3.18 – 10.69). This risk decreases over time and is more prevalent early on, as indicated by a scale value below 1 (0.23).

### Bacteriome

The overall bacterial diversity, as measured by alpha diversity indexes (Shannon, Inverse Simpson, and Chao1), remained similar among AH, HDTL and SH groups, as well as across different ages (from birth to 5 weeks)

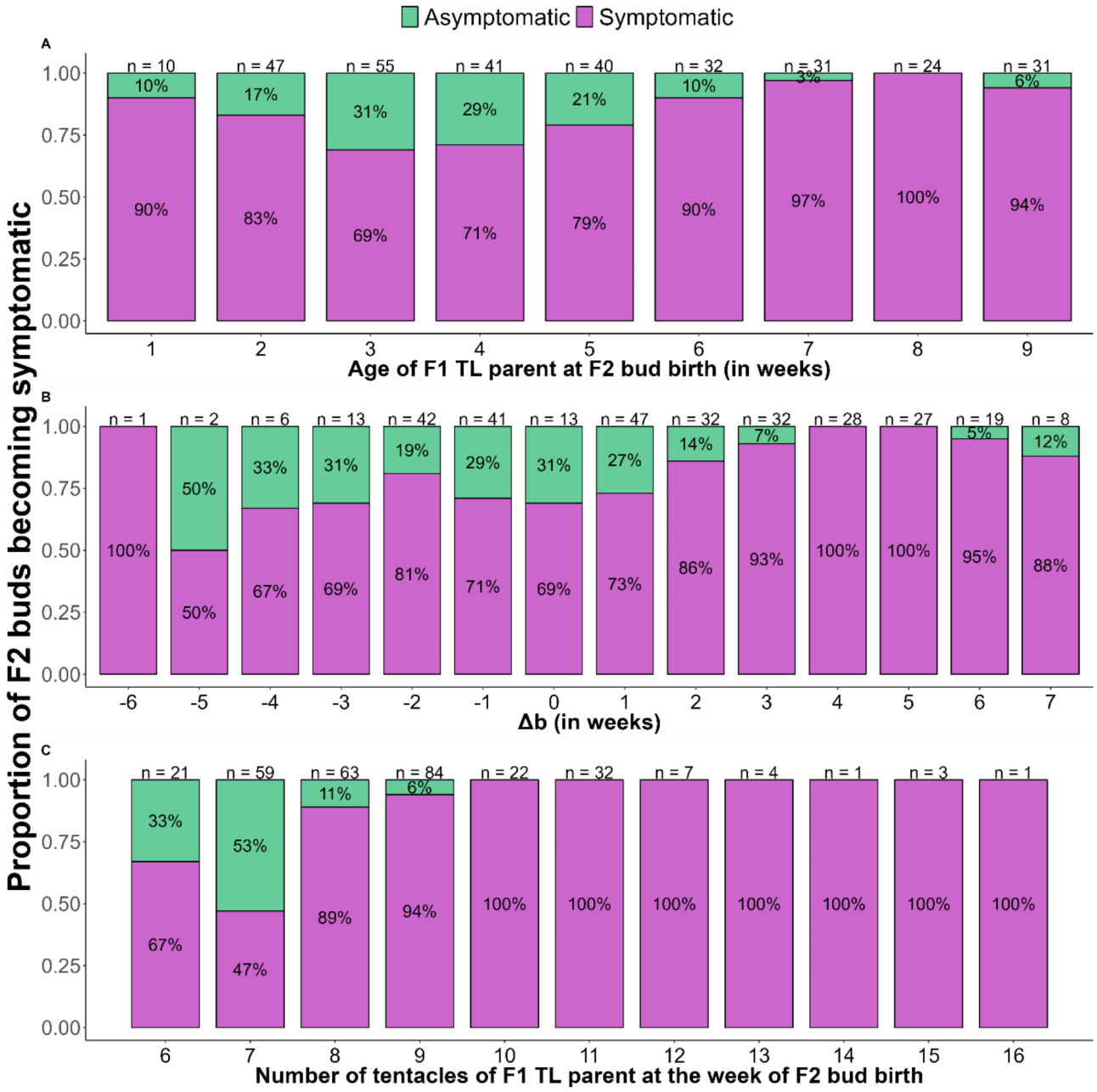


**Fig. 2**. Tumor transmission rate in F1 TH parents. The bar plots represent the proportion of F2 buds that become symptomatic in relation to (**A**) the age of their F1 parent at F2 bud's birth, (**B**) the Δb variable (in weeks), where x = 0 corresponds to the date of tumor onset in the parent (negative values indicate the number of weeks before tumor onset, and positive values correspond to the number of days after tumor onset) and (**C**) the number of tentacles of their F1 parent at the week of the F2 bud's birth. Asymptomatic buds are represented in green, and symptomatic buds in purple.

(Fig. 6A and B, Kruskal-Wallis test, p-value > 0.05). However, when analyzing hydra tentacle number and its impact on bacterial diversity, individuals with −7 tentacles exhibited higher bacterial richness compared to those with 8 + tentacles (electronic supplementary material, Figs. S1A and S1B, Chao1 index, Wilcoxon test, p-value < 0.001).

In terms of beta diversity, HDTL exhibited a distinct bacterial community composition compared to AH and SH (Fig. 6A; see also electronic supplementary material, Fig. S2A), Jaccard index, Pairwise Adonis Test, p-value < 0.05). Chlamydiales were detected in 88% of HDTL, compared to 55% in AH and 47% in SH, with significantly higher abundance in HDTL (Dunn's Test, p-value < 0.001), while no significant difference was observed between AH and SH (Dunn's Test, p-value > 0.05). Leptospirales were present in all hydras across the three groups, but showed significantly lower abundance in HDTL (Dunn's Test, p-value < 0.001), with no significant difference between AH and SH (Dunn's Test, p-value > 0.05). Pseudomonadales, while present in only

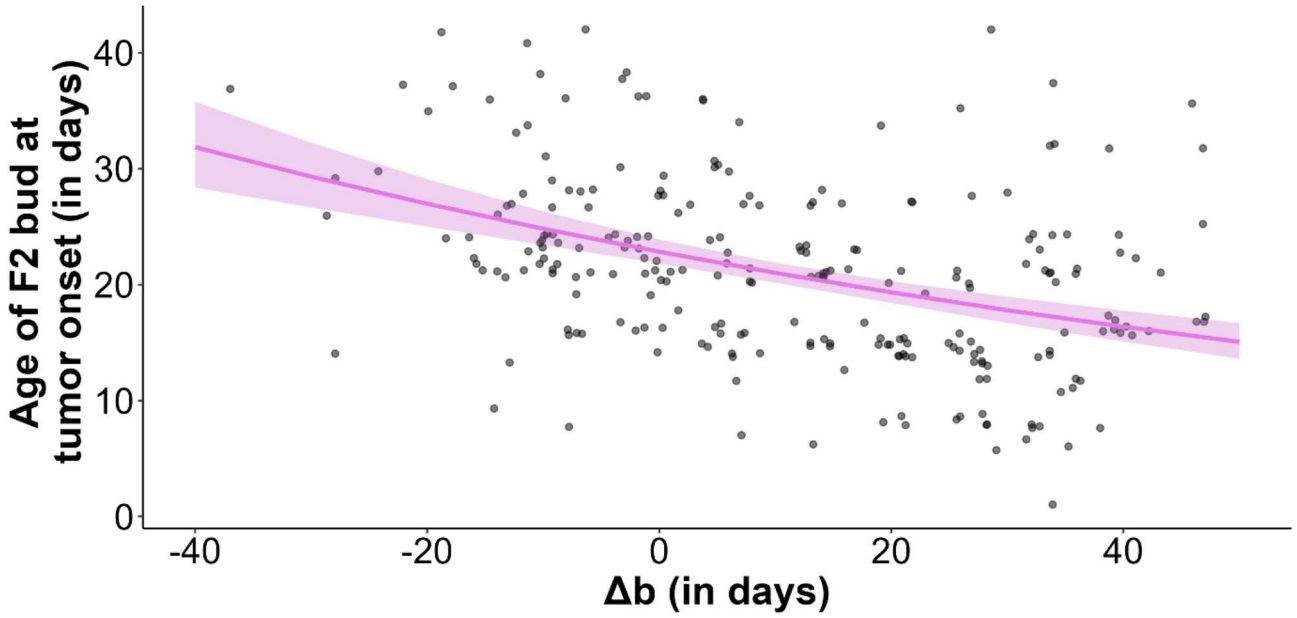


**Fig. 3**. Age of tumor onset in F2 buds. The scatter plot represents the age of tumor onset in F2 buds in relation to Δb variable (in days). Here, x = 0 corresponds to the date of tumor appearance in the F1 parent. Negative values reflect the number of days before tumor onset, and positive values correspond to the number of days after tumor onset in F1 parents. The purple line represents the model-predicted average value along with its 95% confidence interval.

41% of HDTL samples, exhibited a significantly lower abundance (Dunn's Test, p-value < 0.05) compared to AH (71%) and SH (76%), with no significant difference between AH and SH (Dunn's Test, p-value > 0.05).

We also found that bacterial composition did not vary with age for AH and SH. However, HDTL at 5 weeks showed a distinct bacterial composition compared to AH at emergence, 1st, and 2nd weeks, and SH at 2nd, 4th, and 5th weeks (Fig. 6B; see also electronic supplementary material, Fig. S2B), Jaccard index, Pairwise Adonis Test, p-value < 0.05). At week 5, HDTL bacteriome was mainly dominated by Chlamydiales and Leptospirales (Fig. 6B). No significant differences in bacterial composition were found between hydras with −7 tentacles and those with 8 + tentacles, as indicated by the Jaccard dissimilarity (Electronic supplementary material, Fig. S1, Adonis test, p-value > 0.05). We obtained the same conclusions using the Bray-Curtis dissimilarity index (results not shown).

## Discussion

Transmissible tumors represent a rare form of parasitic life, with limited understanding of the biology of their interactions with their host[21,48]. Using the freshwater cnidarian *Hydra oligactis* and its vertically transmissible tumors as a model, we aimed to provide new fundamental insights into this biological interaction.

### Transmission dynamics in TL

A first important result of our study is that TL were found to be infectious to their offspring at any age, i.e., regardless of whether they were in their asymptomatic or symptomatic phases, with no significant differences in tumor transmission between weeks 1 to 9 since birth. Similar findings were described by Boutry et al.[27] regarding hydras in their 2nd (i.e., asymptomatic) and 5th (i.e., symptomatic) weeks since birth. Here, we found comparable transmission rates to those previously reported by Boutry et al.[27], and additionally provided transmission rates for the 1st and 3rd weeks (asymptomatic phases), as well as the 4th weeks, 6th, 7th, 8th, and 9th weeks (symptomatic phases) since birth, which expands our understanding of tumor temporal transmission dynamics in hydras. These results suggest that asymptomatic individuals, although they do not visually present detectable tumors, already harbor and transmit a sufficient quantity of tumor cells to their offspring for them to develop tumors several weeks later. In their previous study, Boutry et al.[27] explored whether the increased budding capacity of hydras before becoming symptomatic was a maximization of immediate reproductive effort (i.e., a compensatory response by the host before tumor development impacts budding capacity) or a manipulation by the tumors to enhance their transmission. Boutry et al.[27] concluded that it was likely an adaptation of the hydra, as the same pattern is observed with sexual reproduction (i.e., gamete production), despite tumors not being transmitted through this reproductive way. Thus, the present results suggest that the selective advantage a hydra could gain from early maximization of its efforts in asexual reproduction (see Introduction and[27]), a priori doesn't result in producing descendants that would remain healthy in the long term (i.e., quality of offspring), but rather in increasing the quantity of offspring before the onset of tumor detrimental effects (i.e., reduced budding and survival).

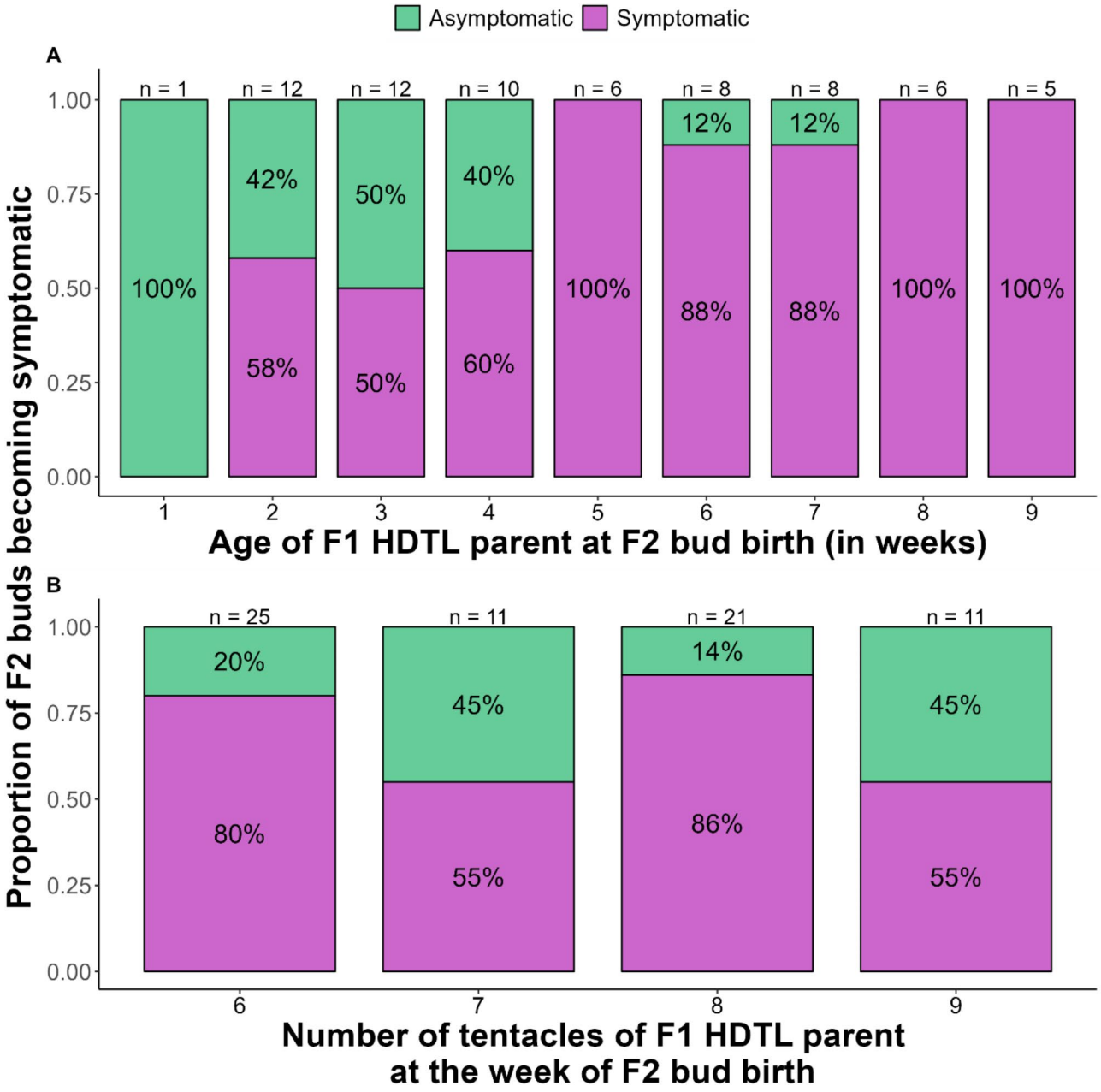


**Fig. 4**. Tumor transmission rate in HDTL F1 parents. The bar plots represent the proportion of F2 buds from F1 healthy parents born from F0 tumoral grandparents that become symptomatic in relation to (**A**) the age of their F1 parent at bud's birth and (**B**) the number of tentacles of their F1 parent at the week of the bud's birth. Asymptomatic buds are represented in green, and symptomatic buds in purple.

Building on these insights into tumor transmission dynamics, our study also uncovered a potential link between the variation in hydra tentacle number and the tumor transmission process. Interestingly, we observed that tumor transmission increased with the number of supernumerary tentacles in the parent, in accordance with the host manipulation hypothesis proposed by Boutry et al.[49]. Previous research has shown that TL with supernumerary tentacles capture more prey compared to their healthy counterparts[5]. Consequently, we hypothesize that TL, by capturing more prey due to an increased number of tentacles, would provide a more abundant nutrient supply, facilitating tumor growth and consequently amplifying tumor cells and their transmission to the new hosts. Moreover, this enhanced feeding may also contribute to the hydras asexual reproductive potential, given the positive correlation between food intake and budding rate in hydras[50]. These findings support the idea that once tumors become transmissible, selection may favor adaptive traits that are usually observed in the world of parasites *sensu stricto*[51], with a similar conclusion reached regarding another transmissible cancer in bivalves (see[19]). As the ability of tumor cells to be transmitted to offspring in *H. oligactis* is a selectable trait[52], it cannot be ruled out that the tumors have been favored by selection to transmit as early

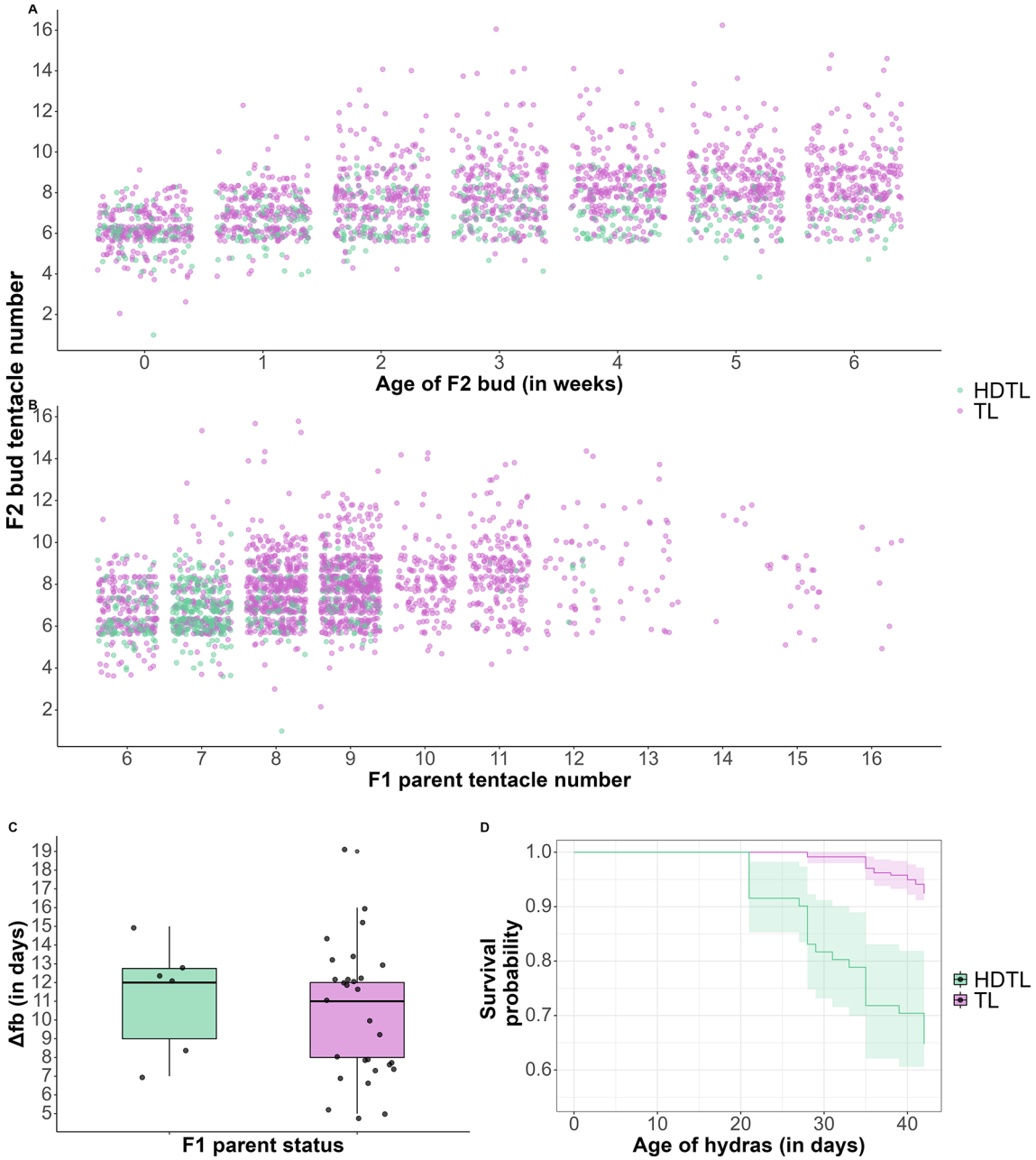


**Fig. 5**. Life-History Traits of HDTL. The scatter plots (A and B) represent relationship between the number of tentacles of F2 buds and (**A**) their age (in weeks, from emergence to week 6) and (**B**) the tentacle number of their F1 parent at birth, between healthy descendants of tumoral line hydras (HDTL) and tumoral line hydras (TL) F2 buds. (**C**) The boxplots represent the relationship between the number of days before first bud appearance and the health status of the F1 parent at the end of experiment (9 weeks), between HDTL and TL F1 parents. (**D**) Kaplan-Meier survival curves represent the survival probability between HDTL and TL, in relation to the age of hydras (in days). HDTL are shown in green, and TL in purple.

as possible, when their host maximizes its early reproductive effort (e.g., exploitation of host compensatory responses, see[53]). However, further research is needed to ascertain the proximate factors behind this phenotypic alteration. Interestingly, this phenomenon also appears to be widespread, as Dujon et al.[54] observed similar tumor formations with the growth of supernumerary tentacles in two independent Australian hydras from river networks (within six months of their transportation to the laboratory). Given these observations, it will be necessary in the future to investigate the timing of supernumerary tentacle appearance - whether it occurs before or after tumor onset - to better understand the sequence of events in tumor-induced phenotypic changes.

Another important finding of this study was that the age of tumor onset in parents had no influence on the age of tumor onset in their offspring, suggesting that this trait is neither genetically inherited across generations (e.g., through heritable genes that predispose hydras to earlier or later tumor onset) nor genetically coded in

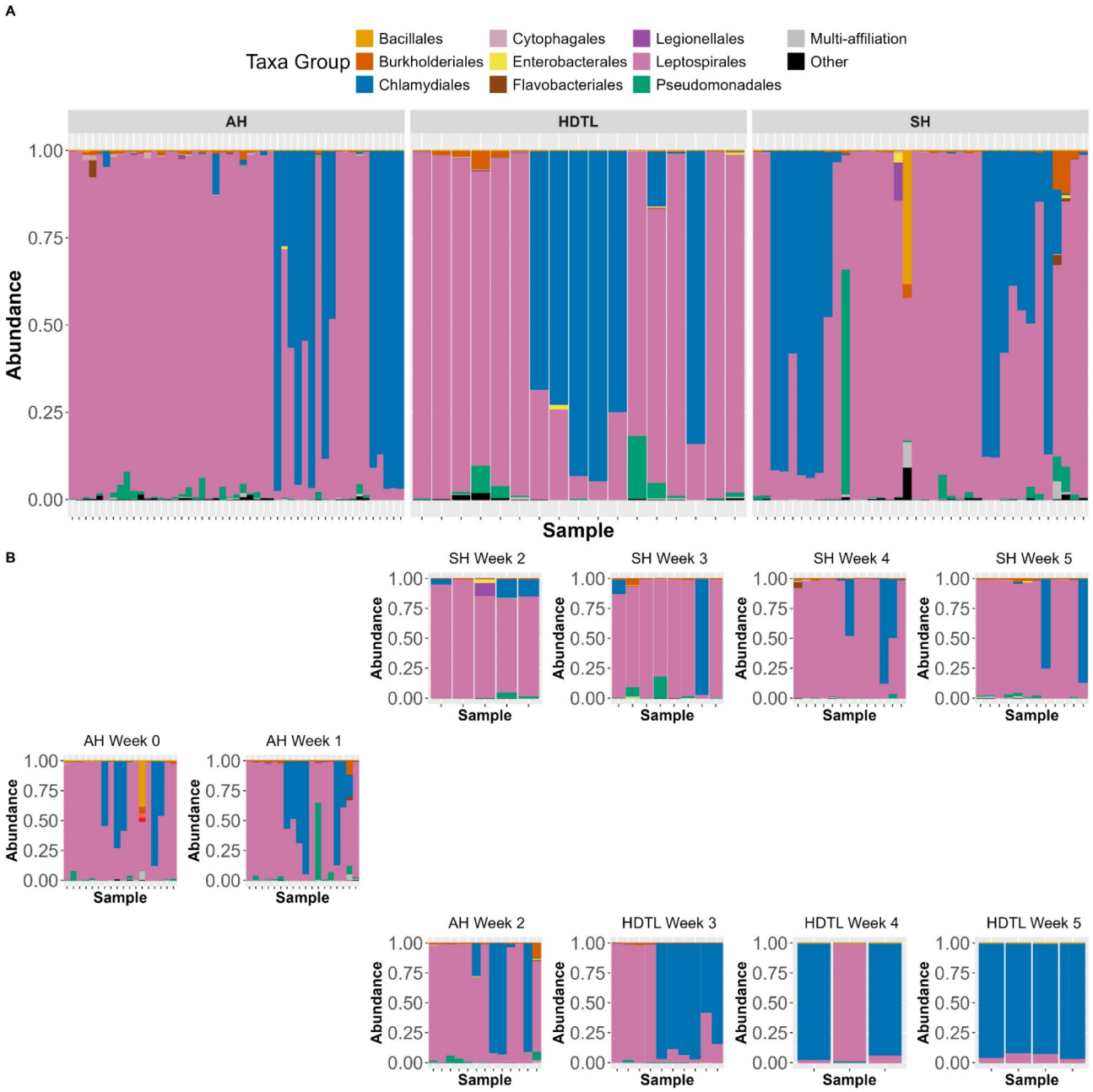


**Fig. 6**. Bacteriome composition between different health statuses and during different weeks. Relative abundance plots showcase the (**A**) overall bacterial composition of asymptomatic hydras (AH), healthy descendants of tumoral line hydras (HDTL), and symptomatic hydras (SH), as well as (**B**) the bacterial composition evolution over 5 weeks. Identified through 16S rDNA sequencing, the plots present the ten most prevalent bacterial orders.

tumor cells (e.g., mutations specific to tumor cells that could influence their growth patterns), and may be influenced by other factors. Interestingly, we also found that tumors developed earlier in offspring as their parent's tumoral progression advanced. One possible explanation is that as hydras harbor increasingly growing tumors, the number of cells in the transmitted tumoral inoculum increases, along with other factors involved in tumorigenesis, thereby accelerating tumor development in the next generation. The size of the inoculum leaving the host is indeed one of the few factors influencing transmission in host-parasite models[55], and potentially in transmissible cancers too (see[56]). For instance, experimental transmission of transmissible cancers, such as BTN, have shown that injecting a larger inoculum into new hosts results in a higher probability of transmission[57].

## Transmission dynamics and life-history changes in HDTL

*Frequency of HDTL appearance*

We provided a clear insight into the occurrence rate of HDTL in both the F1 (17%) and F2 (18%) generations over a 9-week experimental period, underscoring its uncommon occurrence. Prior documentation of HDTL frequency in the literature was scarce. While Boutry et al.[27] presented transmission data for weeks 2 and 5, the discussion did not explicitly address HDTL related issues, and Domazet-Lošo et al.[16], Rathje et al.[26] described them as very rare without providing any quantification, leaving our study as the most comprehensive assessment of HDTL frequency over an extended period. Interestingly, in the case of newly emerged transmissible tumors, a similar prevalence rate of 16% was observed in the fourth generation of the study[52], closely aligning with the rates found in our study. We further explore potential explanations for the uncommon occurrence and asymptomatic phenotype of HDTP observed in our study in the following sections.

*Transmission dynamics in HDTL*

The expression of the tumoral phenotype appears to skip generations, as it occurred in both the F0 and F2 generations, while F1 generation remained asymptomatic (i.e., HDTL). The latter was found to be infectious at any age, from F1 HDTL parents to F2 offspring. To our best knowledge, this study is the first to document this phenomenon in the tumorous Saint Petersburg strain of *H. oligactis*. One potential explanation could be that HDTL harbor tumorigenic factors (i.e., specific bacterial composition), yet there is a delicate equilibrium between factors promoting and inhibiting their expression, thereby sustaining an asymptomatic phenotype. However, for reasons as yet unknown, this equilibrium might be disrupted in their descendants, leading to tumor development. The specific bacteriome composition of HDTL, as shown by Rathje et al., 2020, and also observed in our study, could potentially affect the expression of the tumoral phenotype (see the Bacteriome section). An imbalance in the bacteriome could disfavor the expression of the tumor phenotype, or alternatively, certain bacterial strains within the bacteriome may inhibit tumor development by competing with or suppressing tumor-promoting bacteria. To explore this idea further, investigating the bacteriome composition of tumoral descendants of HDTL is needed in the future. In the context of parasite transmission, it is increasingly demonstrated that the microbiome of parasites plays a crucial role in several aspects of parasite biology[58–60]. Also, the microbiome's variability in arthropod vectors such as mosquitoes and ticks is shaped by environmental factors, host life stage, and sex. This variability can lead to diverse host phenotypes and significantly influence vector competence (parasite transmission) and disease dynamics, for example by affecting pathogen interactions or modulating host immune responses[61,62]. An alternative hypothesis to our results would be that F1 HDTL parents are free of tumoral cells, and that the tumors observed in the F2 offspring are not the result of tumoral transmission but rather spontaneous tumor development. However, we do not favor this hypothesis because the consistent transmission rate observed at any age between the F1 and F2 HDTL generations is not characteristic of patterns usually associated with spontaneous tumor development.

*Biodynamic factors affecting health outcomes in HDTL*

The overall reduced health condition of HDTL is potentially influenced by a variety of factors. For instance, the specific bacteriome composition (see[26] and the Bacteriome section) may compromise their viability and impede the effective expression of the tumoral phenotype, despite the potential presence of tumoral cells. Furthermore, Boutry et al.[27] observed a reduced survival rate in the tumorous Saint Petersburg strain of *H. oligactis*, suggesting that the reduced lifespan of this strain might interact with the altered bacteriome of HDTL in a way that contributes to the particular phenotype of HDTL. In contrast, in the case of newly emerged transmissible tumors, notably the MT40 strain, tumor-free hydras from tumoral parents (TFTP) did not exhibit reduced survival[52]. This could be explained by the fact that, unlike our study, the MT40 strain shows no significant differences in bacteriome composition between tumoral hydras and TFTP, meaning their viability remains uncompromised. Additionally, the lower transmission rate of tumor cells in TFTP (if any) of the MT40 strain, compared to the rate observed in HDTL, suggests that the tumor inoculum is smaller, placing fewer metabolic demands on the host and thus having a more limited impact on survival.

The reduced number of tentacles in HDTL, potentially resulting from hidden developmental abnormalities or their specific bacteriome composition (see[26] and the Bacteriome section), likely impairs their ability to gather sufficient resources, exacerbating the deleterious effects of tumoral cells (if any) and/or of an altered bacteriome. Moreover, decreased feeding efficiency due to fewer tentacles could in itself be responsible for a reduced survival, particularly if resource acquisition is severely compromised. This may lead to fewer resources being allocated to tumoral growth, potentially resulting in a less pronounced tumoral phenotype or even a non-tumoral one. Given that HDTL may harbor tumoral cells, there appears to be a complex interplay between tentacle development, resource acquisition, and tumoral growth. This is supported by a recent study by Boutry et al.[49], which used grafts of tumoral tissue on healthy hydras and showed that tumoral cells are actively manipulating the host to facilitate their own growth, by promoting the development of additional tentacles. We speculate that even in the absence of visible tumors, the presence of tumoral cells or subtle physiological changes may still impose metabolic demands on the host. These demands could potentially lead to increased mortality if the host is unable to acquire sufficient resources to meet both its own needs and those of the tumor cells.

The limited resource acquisition might also explain why there is no significant difference in the age of first reproduction, suggesting that HDTL do not advance their first reproduction age to compensate for future fitness loss. Similar findings were observed regarding the age of first reproduction in both tumoral and healthy Saint Petersburg strain hydras by Boutry et al.[27] as well as in newly emerged transmissible tumors in the MT40 strain between healthy, tumoral and TFTP[52]. This suggests that earlier reproduction does not result in significant fitness gains and therefore is not favored by selection. Furthermore, species can only adjust their life-history traits to a certain extent (see[63]), and hydra may already be at their maximum capacity for such adjustments.

Alternatively, HDTL, potentially burdened by tumoral cells and by a compromised microbiome (see Bacteriome section), may be unable to optimize their reproductive timing. Lastly, the association between tentacle numbers in F1 parents and F2 buds for both HDTL and TL suggests that tentacle number tends to be inherited across generations, though with some variability.

## Bacteriome

We found no significant changes in microbial diversity trajectory before and after tumor development in hydras, maintaining a microbial composition and abundance similar to that observed at advanced tumoral stages from the earliest phases of bud emergence. This aligns with consistent tumor cell transmission before and after tumoral development. Interestingly, while Leptospirales were consistently detected in all individuals, Pseudomonadales, whose interaction with Leptospirales was deemed essential for tumor initiation[26], were not universally present. Additionally, HDTL, which demonstrated potential infectiousness at any stage, lacked a typical tumor-specific bacteriome composition. In the case of transmissible tumors in other strains, such as the newly emerged MT40 or *Pelmatohydra robusta* strains, these tumors intriguingly do not appear to be associated with any specific bacteriome[26,52]. This raises questions about the bacteriome's direct role in tumor initiation, suggesting that its influence might not be causative but rather supportive in the proliferation of the tumor to the point of being visible. For example, in the case of human colorectal carcinoma, *Fusobacterium nucleatum* has shown to promote colorectal tumor growth and inhibit T cell-mediated immune responses against colorectal tumors[64].

Furthermore, Chlamydiales (first described in hydra by Boutry et al.[31]) were detected in most samples, particularly abundant in HDTL, especially in those at their fifth week of life, where they, along with Leptospirales, appeared to dominate, with no presence of Pseudomonadales or other bacteria. Interestingly, Pseudomonadales were not entirely absent in HDTL overall. This finding contrasts with Rathje et al.[26], who reported a spontaneous loss of Pseudomonadales in HDTL. Due to the lack of detailed information regarding the age and sample size in Rathje's study, we cannot exclude the possibility that their observations might pertain to older individual. It is also possible that HDTL presenting Pseudomonadales in their bacteriome in our study were actually AH that would eventually develop a tumoral phenotype. Also, it remains possible that the observed differences with Rathje et al.[26] may be influenced by slightly different hydra culture conditions (e.g., in our experiment, hydras were fed three times a week compared to two times a week in Rathje et al.[26]). In addition, given that culture conditions could also affect fungal communities, it would be worthwhile, in future studies, to investigate whether fungal interactions contribute to the observed variations in the bacteriome, including potential influences from the F0 generation.

The limited presence of Pseudomonadales in HDTL at their fifth week could possibly be explained by the competitive dynamics within the environment. Chlamydiales, found in the endoderm[31], and Leptospirales as well as Pseudomonadales, located in the mesoglea[26,31], may exert indirect effects on the bacteriome. Despite occupying distinct niches, Chlamydiales presence could alter the overall bacterial balance, potentially providing them with a competitive advantage and leading to the replacement or reduction of Pseudomonadales and other bacteria in HDTL bacteriome. For instance, similar competitive interactions have been observed in other contexts, such as human colorectal cancer (CRC), where CRC-associated metabolic and physiological changes would recruit tumor-foraging commensal-like bacteria, presenting a competitive advantage in the tumor microenvironment and replacing the pathogenic bacteria that may be implicated in CRC etiology[65]. Alternatively, HDTL in our study could represent individuals that did not inherit the proper bacteriome composition necessary for promoting a tumorous phenotype or for being healthy, which may also explain their survival rate. This "bad bacteriome" could promote the proliferation of other bacteria, such as Chlamydiales. For further studies, the correlation between tumoral phenotype expression/tumoral cell abundance and the ratio of Chlamydiales, Pseudomonadales and Leptospirales in these individuals needs to be explored. This will involve analyzing the bacteriome of individuals with varying intensities of tumoral phenotype expression (from stage 0 to stage 6, see[28]). Additionally, following the same protocol as in Rathje et al.[26] we propose injecting Chlamydiales into hydra at different proportions and observing the resulting changes in tumoral phenotype expression/cell abundance. This approach will help us better understand the potential causal relationships and underlying mechanisms between these bacterial orders and tumoral development.

Finally, we found that the presence of supernumerary tentacles in hydras is associated with a lower bacterial richness, while showing no difference in bacterial composition. The lack of differences in microbial composition between hydras with and without supernumerary tentacles is somewhat unexpected. One might have assumed that the altered microenvironment created by the supernumerary tentacles could lead to distinct bacterial communities. The fact that this is not the case suggests that although the overall bacterial richness may be affected by the presence of supernumerary tentacles, the specific taxa constituting the bacterial community remain relatively stable. This observation hints at the possibility that certain bacterial taxa exhibit greater resilience or adaptability to changes in the hydra's physiology compared to others, thereby maintaining a consistent presence regardless of external factors such as the presence of supernumerary tentacles. Additionally, the reduced richness of bacterial communities in hydras with supernumerary tentacles may indicate that the microenvironment generated by the supernumerary tentacles could selectively favor certain bacterial species over others. These favored species may possess traits that make them more competitive (e.g., faster reproduction rates, metabolic flexibility) and enable them to occupy similar ecological niches, consequently diminishing the abundance of less competitive ones. Similarly, in some diseases, such as cystic fibrosis (CF), the thick mucus and nutrient-rich environment of the CF lungs create a breeding ground where opportunistic pathogens can outcompete other bacteria, leading to dominance and exacerbation of the disease symptoms[66].

To enhance our comprehension of the ecological interactions between hosts, tumors, and their associated bacteriomes in hydra, additional research could delve into the long-term effects of supernumerary tentacles on

the hydra's bacteriome. This research could also aim to elucidate the mechanisms that contribute to the stability of microbial composition over time.

## Conclusion

This study provides new insights into the transmission dynamics of tumors in *Hydra oligactis*, a rare model of vertically transmissible tumors. We found that TL can transmit tumoral cells to their offspring at any age, whether in asymptomatic or symptomatic stages, with no significant differences in transmission rates. This suggests that even AH harbor and transmit sufficient tumor cells to initiate tumoral formation in their offspring, indicating that these tumors act similarly to parasites by exploiting their host for transmission and propagation. Furthermore, the association between a higher number of tentacles and increased tumoral transmission supports the hypothesis of host manipulation by the tumor, promoting its own growth and spread.

We also found that while the age of tumor onset in parents did not directly influence the age of tumoral onset in their offspring, tumors appeared earlier in offspring when the parent's tumoral progression was more advanced. This suggests that the size of the tumoral inoculum—the quantity of tumor cells transmitted—likely increases as the parent's tumor develops, thereby accelerating tumoral growth in the next generation. This is consistent with models of host-parasite transmission, where a larger infectious inoculum leads to higher transmission efficiency and faster disease progression.

Regarding HDTL, we observed that these individuals exhibit distinct life-history traits, such as reduced lifespan and fewer tentacles. However, they remain capable of transmitting tumor factors to their progeny, suggesting a delicate balance between the presence of tumoral cells and the mechanisms inhibiting their phenotypical expression.

Furthermore, while no significant differences in bacterial composition were found between AH and SH, those with supernumerary tentacles exhibited reduced bacterial richness. This suggests that certain tumor-driven phenotypic changes may affect the bacterial community structure, potentially influencing tumoral dynamics and transmission.

The novelty of our research lies in demonstrating that tumors can be transmitted vertically across multiple generations, even when hosts are asymptomatic at any age, and in identifying the biological factors that may influence this process, such as parental age at tumor onset (which had no effect), the size of the tumoral inoculum, the presence of supernumerary tentacles and the composition of the bacteriome. These findings expand our understanding of host-transmissible tumor interactions, broadening our view of tumors as parasitic infectious agents. Lastly, this study provides a foundation for future research, particularly on the mechanisms of host manipulation by tumors and the role of the bacteriome in tumoral phenotype expression and transmission.

## Data availability

Scripts and data associated are provided in electronic supplementary material. The 16S rRNA raw sequence files for this study have been deposited in FASTQ format and can be found in the Sequence Read Archive from NCBI (BioProject: PRJNA1203433).

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N.S.: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing—original draft, writing—review and editing; M.P.: formal analysis, investigation, visualization, writing—original

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## Declarations

## Competing interests

The authors declare no competing interests.

## Additional information

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