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1 # Effects of parental care on skin microbial community
2 # composition in poison frogs
3

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26 # Abstract

27 Parent-offspring interactions constitute the first contact of many newborns with their environment,
28 priming community assembly of microbes through priority effects. Early exposure to microbes can have
29 lasting influences on the assembly and functionality of the host's microbiota, leaving a life-long imprint on
30 host health and disease. Studies of the role played by parental care in microbial acquisition have primarily
31 focused on humans and hosts with agricultural relevance. Anuran vertebrates offer the opportunity to
32 examine microbial community composition across life stages as a function of parental investment. In this
33 study, we investigate vertical transmission of microbiota during parental care in a poison frog (Family
34 *Dendrobatidae*), where fathers transport their offspring piggyback-style from terrestrial clutches to aquatic
35 nurseries. We found that substantial bacterial colonization of the embryo begins after hatching from the
36 vitelline envelope, emphasizing its potential role as microbial barrier during early development. Using a
37 laboratory cross-foster experiment, we demonstrated that poison frogs performing tadpole transport serve
38 as a source of skin microbes for tadpoles on their back. To study how transport impacts the microbial skin
39 communities of tadpoles in an ecologically relevant setting, we sampled frogs and tadpoles of sympatric
40 species that do or do not exhibit tadpole transport in their natural habitat. We found more diverse microbial
41 communities associated with tadpoles of transporting species compared to a non-transporting frog.
42 However, we detected no difference in the degree of similarity between adult and tadpole skin microbiotas,
43 based on whether the frog species exhibits transporting behavior or not. Using a field experiment, we
44 confirmed that tadpole transport can result in the persistent colonization of tadpoles by isolated microbial
45 taxa associated with the caregiver's skin, albeit often at low prevalence. This is the first study to describe
46 vertical transmission of skin microbes in anuran amphibians, showing that offspring transport may serve as
47 a mechanism for transmission of parental skin microbes. Overall, these findings provide a foundation for
48 further research on how vertical transmission in this order impacts host-associated microbiota and
49 physiology.

50 # Introduction

51 Host-associated microbial communities contribute to health and facilitate ecological adaptations by
52 playing critical roles in host development, nutrition, pathogen exclusion, and immune response (Belkaid &
53 Hand, 2014; Donald & Finlay, 2023; Gensollen et al., 2016; Mazmanian et al., 2005; Reynolds & Bettini,
54 2023; Sampson & Mazmanian, 2015; Von Frieling et al., 2018). Across host species, early exposure to
55 microbes can exert lasting influences on the assembly and functionality of the host's microbiota (Al
56 Nabhani & Eberl, 2020; Powell et al., 2014; Warne et al., 2017; Zepeda Mendoza et al., 2018). Timing of
57 microbial contact is particularly critical in newborns and can prime community assembly through priority

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58 effects, where species that arrive early impact establishment of later colonizers (Debray et al., 2022;
59 Fukami, 2015; D. Sprockett et al., 2018). In many species, the transmission of host-adapted microbes is
60 facilitated by parental care strategies, such as feeding, grooming and direct skin contact (for review see e.g.
61 S. Wang et al., 2020 for humans, Klug & Bonsall, 2014 for animals; see also Blyton et al., 2022; D. W.
62 Chen & Garud, 2022; Pascoe et al., 2017; D. D. Sprockett et al., 2020; Sylvain & Derome, 2017). To date,
63 most studies investigating effects of exposure to microbiota during parental care center on humans and
64 livestock, while vertical transmission remains largely unexplored in other classes of vertebrates (but see
65 Kouete et al., 2023). Therefore, investigating early-life microbial colonization in other species that have
66 dedicated parenting strategies offers opportunities to understand microbe-host interactions in a wider
67 variety of ecologically relevant contexts.

68 Acquisition of host-adapted skin microbes is especially important in amphibians, which rely on
69 healthy skin for physiological processes such as respiration, osmoregulation, immune response and barrier
70 function (Çömden et al., 2023; Duellman & Trueb, 1986; Varga et al., 2019). The amphibian skin
71 microbiota also serves to exclude pathogens, as the skin microbiome can confer resistance to infections by
72 the deadly chytrid fungus *Batrachochytrium* (Alexiev et al., 2023; Harris et al., 2006, 2009; Loudon et al.,
73 2014; Vredenburg et al., 2011; Woodhams et al., 2007), which decimates amphibian populations worldwide
74 (Daszak et al., 2003; Luedtke et al., 2023; Wake & Vredenburg, 2008). Many studies have characterized
75 microbe-host interactions in adult frogs in the context of chytrid infections, but few have focused on
76 microbiomes associated with tadpoles (Fontaine et al., 2022; Santos et al., 2023; Warne et al., 2017, 2019;
77 Weinfurther et al., 2023). Those studies that have looked at tadpoles have primarily characterized the
78 changes in bacterial communities across development and metamorphosis (discussed e.g. in Hughey et al.,
79 2017; Kueneman et al., 2014; Prest et al., 2018; Warne et al., 2017, 2019). Furthermore, most of this
80 research has focused on temperate-region, pond-spawning species, where opportunities to investigate
81 effects of parental care on community assembly are limited. These species generally lay many eggs at once
82 into bodies of water to avoid desiccation and larvae develop without further parental contact or care. In the
83 tropics, a warm and humid climate has favored the progression from aquatic to terrestrial reproduction,
84 where clutches are laid on land and parents then care for their offspring (McDiarmid & Altig, 1999; Wells,
85 2007). Neotropical frogs show more diverse reproductive strategies where parents construct foam nests,
86 attend clutches, defend eggs against predators, and guard, transport or feed their tadpoles (reviewed e.g. in
87 Schulte et al., 2020, see also Crump, 2015; Delia et al., 2013; Requena et al., 2009; Warkentin, 1995).
88 Priority effects influence community assembly of frog embryos (K. R. Jones et al., 2023, 2024) but previous
89 studies have found no direct evidence for vertical transmission of microbes during egg attendance (Hughey
90 et al., 2017). However, this form of offspring care does not involve direct contact between the parents and
91 offspring as developing tadpoles in the clutch are surrounded by several layers of gelatinous jelly and a

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92 double vitelline envelope before they hatch (see Altig & McDiarmid, 2007 and Méndez-Tepepa et al., 2023
93 for a review). While the vitelline layer may serve as an antimicrobial barrier in chickens (Guyot et al., 2016;
94 Mann, 2008), studies of its role in microbial colonization of anuran embryos are currently lacking.

95 Parental care involving skin-to-skin contact has developed multiple times independently in
96 Neotropical dendrobatoid frogs (Superfamily *Dendrobatoidea*), commonly referred to as poison frogs
97 (Grant et al., 2017; Weygoldt, 1987). Closely related species in this clade display a variety of parenting
98 behaviors ranging from egg attendance and transport of hatched tadpoles to provisioning larvae with
99 unfertilized eggs (Crump, 1974; Ringler et al., 2023; Wells, 2007). The most widespread form of care
100 beyond clutch attendance is tadpole transport, where parents shuttle hatched tadpoles piggy-back style from
101 terrestrial clutches to water pools (Furness & Capellini, 2019). Transport can last multiple hours to days,
102 where tadpoles adhere tightly to the backs of their caregivers (Pašukonis et al., 2019; Pröhl & Berke, 2001;
103 Ringler et al., 2013, p. 201; Wells, 1980). Therefore, poison frogs provide the opportunity to evaluate the
104 effects of parental care on offspring skin microbial community assembly. The adaptive value of parental
105 care in this clade has received considerable attention (reviewed in Schulte et al., 2020; Summers & Tumulty,
106 2014), but how reproductive strategies involving skin-to-skin contact impact microbial colonization of
107 tadpoles and whether these effects persist to later developmental stages has, to our knowledge, not been
108 studied in any anuran to date.

109 The variable poison frog *Ranitomeya variabilis (Rv)* (Zimmermann & Zimmermann, 1988) is
110 particularly well-suited to study vertical transmission of microbes during tadpole transport. This small
111 species is diurnal, commonly found across South America and readily reproduces in captivity. Males of this
112 species often assist their larvae with hatching (Brown et al., 2008) and carry tadpoles on their back for up
113 to 48 hours (Lötters et al., 2007). The cannibalistic tadpoles grow up in individual pools where they feed
114 on algae and leaf debris until they complete their development after about five months. In the natural study
115 population located in the reserve 'Les Nouragues' in French Guiana, these frogs live on rocky outcrops, use
116 bromeliads as a resource for reproduction and shuttle tadpoles from the oviposition site in arboreal plants
117 to water reservoirs in leaf axils of terrestrial bromeliads (Poelman et al., 2013; Poelman & Dicke, 2007,
118 2008; Sarthou, 2001). This species is sympatric with other anuran species with differing parenting
119 strategies: the poison frog *Allobates femoralis* (*Af*) shuttles tadpoles to water but transporting periods are
120 shorter and tadpoles live in groups. The leptodactylid frog *Leptodactylus longirostris* (*Ll*) deposits eggs in
121 ephemeral rock pools and does not exhibit parental care. These cohabiting species are potentially useful for
122 determining if reproductive strategies impact patterns of microbial diversity of tadpoles.

123 In this study, we combine laboratory experiments with sampling of anuran populations in the wild
124 to examine vertical transmission of microbes during tadpole transport and address implications for

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community structure across life stages. In the laboratory, we investigated microbial colonization of hatched and unhatched *Rv* embryos and then tested the hypothesis that transporting frogs serve as a source for bacteria on tadpole skins using cross-foster experiments. We complemented these experiments with a comparative field study examining microbiomes associated with tadpoles and adults of three anuran species with varying parenting behaviors to examine how shuttling affects community composition on the skin of tadpoles in their native habitat. To our knowledge, this is the first study to document and characterize vertical transmission of microbes during parental care in any anuran.

Results

Microbial colonization of a poison frog embryo occurs after hatching

We used non-sterile frogs of our captive laboratory colony to determine the timing of microbial colonization of poison frog embryos. Embryos within the vitelline membrane exhibit a characteristic C-shaped posture due to the membrane's restriction on their movement and adopt a straight posture after hatching (Fig. S1A). First, we qualitatively examined bacterial presence in *Rv* egg jellies and embryos across tadpole developmental stages using a broad range PCR targeting the 16S rRNA gene. We manually separated embryos in the vitelline envelope from the surrounding jelly and processed each for DNA extraction (Fig 1A). Bacteria were detected in egg jellies across all developmental stages (formation of the dorsal lip to hatchling) but were not detected in embryos prior to hatching from the vitelline membrane (N = 12) (Fig 1B, Fig. S1B). After hatching, bacteria were detected in tadpoles that remained in the jelly and in tadpoles that were transported by their caregiver (Fig. 1B, Fig. S1B). Next, we confirmed differences in bacterial load between jelly and embryo by examining variations in 16s rRNA copy numbers using a droplet digital PCR (ddPCR) approach suited for low biomass samples (Abellan-Schneyder et al., 2021). We found that unhatched embryos contained on average 2942 copies of the 16S rRNA gene per µl (min = 742, max = 11584, std = ±3086) while jellies contained on average 6128 times as many (min = 457417, max = 66115451, mean = 18029604 ±18586471; Kruskal Wallis (KW): chi-squared = 18.6667, df = 2, $p < 0.00001$) (Fig. S1C, Table S1). 16S rRNA gene copy concentrations were on average 8310 times higher in hatched relative to unhatched embryos (min = 737340, max = 48159335, mean = 24448337 ±33532414; KW: p = 0.0195), but did not differ significantly from concentrations detected in the jelly (KW: p = 0.5) (Fig. S1C, Table S1).

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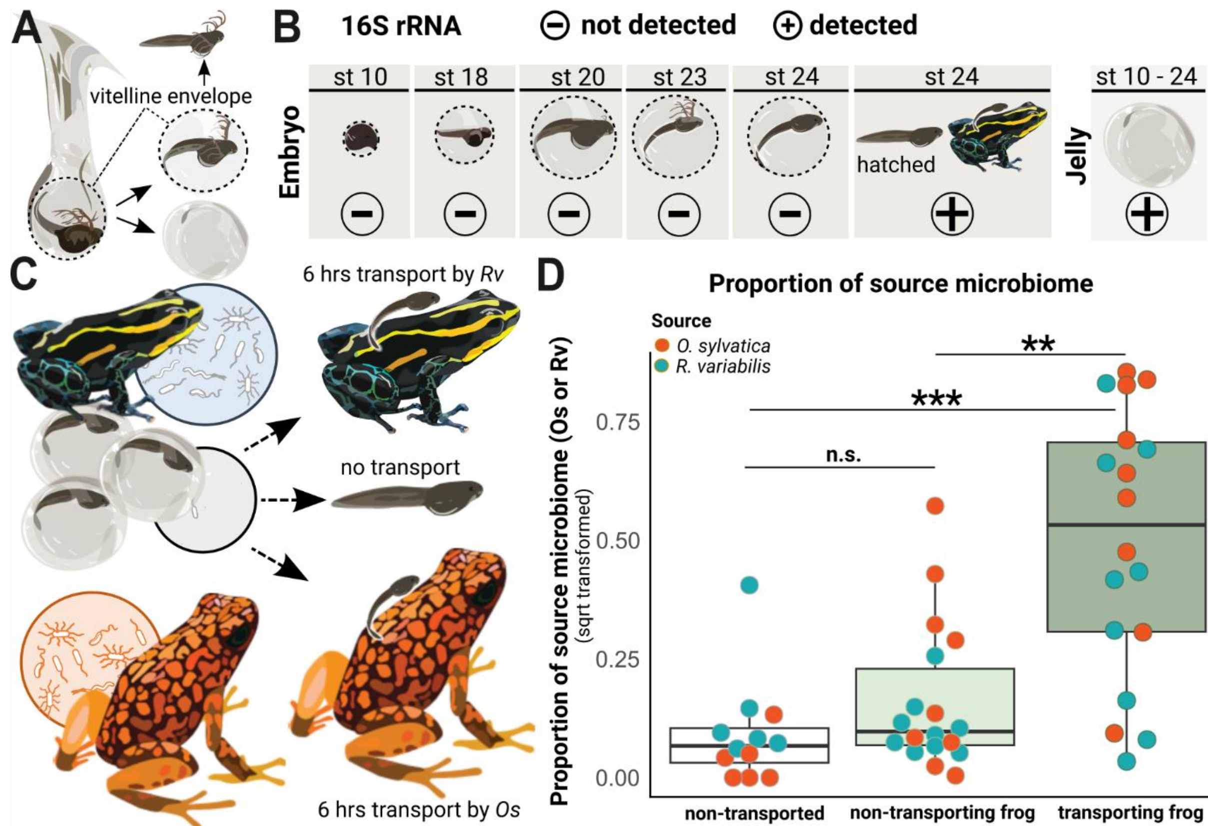

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154 **Figure 1**. **Microbes colonize a poison frog embryo after hatching and are vertically transmitted during tadpole transport.**
155 **(A)** Embryos in the vitelline envelope (dashed line) were manually separated from jelly using tweezers. The vitelline envelope
156 (dashed line) containing the embryo was transferred to sterile water and opened to free the embryo. The embryo was washed in
157 fresh sterile water before homogenization. **(B)** Detection/non detection of 16S rRNA across developmental stage. DNA was isolated
158 from whole embryos and jellies of different developmental stages and tested for bacterial presence using a broad range PCR for
159 near-complete 16S rRNA gene (see also Fig. S1). **(C)** After hatching, siblings of a clutch were either (1) not transported (middle
160 arrow), (2) transported by their biological parent (upper arrow), or (3) transported by a foster poison frog of a different species
161 (*Oophaga. sylvatica*) (lower arrow). **(D)** We performed 16S v4 specific amplicon sequencing on swabs from the transporting frogs
162 and the skins of the transported tadpoles and used Sourcetracker to identify the sources of taxa that had been acquired by tadpoles.
163 The function was trained on communities of adult *Rv* and *Os* that had served as caregivers. Source proportions of both species (*Os*:
164 orange dots and *Rv*: blue dots) were determined for each tadpole (N = 24), resulting in 2 data points per tadpole. Proportions were
165 then grouped to display either (1) proportions of the transporting species in transported tadpoles (*Rv* proportions in tadpoles
166 transported by *Rv* and *Os* proportions in tadpoles transported by *Os*) (dark green), or (2) proportions of the non-transporting species
167 on transported tadpoles (indicating *Rv* proportions in tadpoles transported by *Os* and *Os* proportions in tadpoles transported by *Rv*)
168 (light green), or (3) proportions of both species in non-transported tadpoles (indicating *Rv* and *Os* proportions in non-transported
169 tadpoles) (white). Proportions were compared with a Kruskal-Wallis test with Benjamini-Hochberg correction. Source proportions
170 were square root transformed for plotting.

171 Evidence for vertical transmission of microbiome during parental care in laboratory-reared poison frogs

172 We used a cross-foster design to test the hypothesis that transporting frogs serve as a source of
173 microbes for communities on tadpole skin. Siblings of the same clutch (N = 6 clutches from 2 *Rv* pairs)
174 were randomly assigned to three groups: (1) not transported (N = 6 tadpoles), (2) transported by their
175 biological parent for six hours (N = 9 tadpoles), or (3) transported by a heterospecific poison frog (*Oophaga*
176 *sylvatica, Os*) for six hours (N = 9 tadpoles) (Fig. 1C). For this design, sequence-based surveys of amplified
177 16S rRNA genes were used to assess the composition of skin-associated microbial communities from

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178 tadpoles and their adult caregivers (the tadpole-transporting frogs). We found that bacterial community
179 composition on caregiver skin was primarily shaped by host species (*Rv* versus *Os*; Adonis: Bray-Curtis:
180 $F_{1, 12} = 5.6538$, $R^2 = 0.33949$, p = 0.001; unweighted Unifrac: $F_{1, 12} = 2.0078$, $R^2 = 0.15435$, p = 0.013),
181 allowing us to distinguish between these potential sources of tadpole-colonizing microbes (Fig. S2A).
182 Indeed, we found that after six hours of transport, bacterial community composition on *Rv* tadpole skin was
183 influenced by caregiver species (*Rv* versus *Os*; Adonis: Bray-Curtis: $F_{1, 12} = 1.75$, $R^2 = 0.099$, p = 0.017;
184 Unifrac: $F_{1, 12} = 1.67$, $R^2 = 0.095$, p = 0.015). Despite their distinct compositions, we observed substantial
185 overlap between communities of tadpoles transported by *Os* and *Rv* (Fig. S2B), possibly reflecting
186 similarities in tadpoles' skin communities arising from a shared clutch environment after hatching until
187 transport.

188 We used 'Sourcetracker' (Knights et al., 2011) to determine the relative contribution of the
189 transporting frogs as a source of bacteria detected in tadpoles' skin after six hours of transport. Transported
190 tadpoles (N = 18) shared higher proportions of their skin communities with the transporting species than
191 did non-transported tadpoles (N = 6) (Kruskal Wallis $chi^2 = 19.4029$, df = 2, p = 0.0001) (Fig. 1D).
192 Similarly, all but two transported tadpoles shared a larger proportion of their communities with the
193 transporting species than with the non-transporting species (p = 0.001) (Table S2). Tadpoles of a clutch that
194 were transported by *Os* always shared a higher proportion of their communities with this heterospecific
195 caregiver than siblings that were not transported (KW: $chi^2 = 14.4696$, df = 2, p = 0.0003; difference in
196 proportion: median = 39.33, min = 0.872 %, max = 68.478 %) or siblings transported by the biological
197 parent (KW: p = 0.015; percentage of higher proportion: median = 37.07 %, min = 0.874 %, max = 72.97
198 %) (Fig. S2C, Table S2). Non-transported tadpoles were colonized by between 5 and 57 Amplicon
199 Sequence Variants (ASV) (median = 12). We conducted a separate analysis defining non-transported
200 tadpoles as potential microbe source to represent community proportions that were acquired in the clutch
201 before caregiver contact (Fig. S2D). We found that transported tadpoles shared between 0 and 46.99 %
202 (median = 13%) of their communities with non-transported tadpoles. Sourcetracker detected higher
203 proportions of clutch-acquired microbes in tadpoles transported by *Os* (pairwise Wilcoxon test, p = 0.02).
204 This likely reflects the higher degree of similarity between the source clutch and *Rv*, as the clutch remained
205 in the *Rv* tank during development, making it harder to distinguish between these sources. Overall, our
206 results suggest that microbes are vertically transmitted during tadpole transport in a poison frog.

207 <u>Tadpoles of the poison frogs *Ranitomeya variabilis* and *Allobates femoralis* host more diverse skin</u>
208 <u>communities than tadpoles of the leptodactylid frog *Leptodactylus longirostris*.</u>

209 To investigate how vertical transmission of microbes during tadpole transport affects microbial
210 skin communities of tadpoles in an ecologically relevant context, we studied the skin microbiome

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211 composition and diversity of *Rv* tadpoles and adults in a natural population. To assess the broader relevance
212 of our findings to frog species that transport their tadpoles, we also sampled two sympatric species with
213 differing reproductive strategies: *Allobates femoralis (Af)* and *Leptodactylus longirostris* (*Ll*) (Fig. 2A). In
214 contrast to *Rv* that transports its cannibalistic tadpoles to individual pools in bromeliads, *Af* lives in forest
215 leaf litter and shuttles multiple non-cannibalistic tadpoles to the same pool where they grow up
216 gregariously. *Ll* is a nocturnal leptodactylid frog inhabiting the rock savanna that does not exhibit tadpole
217 transport but deposits eggs directly in ephemeral rock pools where the larvae grow up together (Fig. S3).

218 We compared bacterial communities from 137 skin swabs of adult frogs, tadpoles, and the aquatic
219 environment belonging to the tadpoles of these three species. We sampled 44 adults and 21 tadpoles (Gosner
220 stages 29 - 41, categorized as "medium" and "large") of the species *Rv*, ten adults and 14 tadpoles (Gosner
221 stages 34 - 41, categorized as "medium" and "large") of the species *Ll*, and ten adult and eight tadpoles
222 (Gosner stage 25 - 26, categorized as "small") of the species *Af* (Table S3). Additionally, we monitored the
223 growth of 184 *Rv* tadpoles in separate bromeliad pools for up to 82 days. Out of 45 tadpoles that were
224 monitored for over 50 days, 13 (three "small" and ten "medium") tadpoles did not advance in their sizing
225 category, reflecting that the complete larval developmental in this population spans multiple months.
226 Cannibalistic *Rv* tadpoles grow up in isolated pools that were sampled as single replicates when collecting
227 the tadpole. Due to their development in groups, *Af* and *Ll* tadpoles were collected from fewer water bodies
228 and represent a narrower developmental window (stages 34 to 41 for *Ll* and stages 25 and 26 for *Af*). If
229 multiple tadpoles were collected from the same aquatic environment (*Af* and *Ll* tadpoles), we sampled
230 triplicates of the respective environment.

231 For each species, the aquatic environment of the tadpoles displayed a higher average phylum-level
232 diversity than the inhabiting tadpoles or adults (Table S3). As previously reported for Neotropical frogs
233 (Hughey et al., 2017; Kueneman et al., 2016), ASVs belonging to Phylum Proteobacteria dominated the
234 microbial community across all species and life stages (mean relative abundance: 70.9%), followed by
235 Bacteroidota (10.3%), Actinobacteriota (4.1%), Firmicutes (4%), Verrucomicrobiota (2.3%) and
236 Planctomycetota (1.1%) (Fig. S4). The abundance of the frog pathogen *Batrachochytrium dendrobatidis*
237 (*Bd*) in the rock savanna was low, with detection in two samples of *Rv* adults and one *Rv* tadpole, but no
238 positive *Ll* sample. We detected *Bd* on one adult *Af* individual collected in primary forest but not on tadpoles
239 of this species collected in the same area. In all species, tadpole communities contained fewer taxa with
240 known *Batrachochytrium*-growth inhibiting function than did environmental samples or adults (Table S3).

241 We found no significant differences in the observed ASV richness, diversity (Shannon), or
242 evenness between tadpoles and adults of the poison frogs *Rv* and *Af*. In contrast, *Ll* tadpoles were
243 significantly less diverse than *Ll* adults (Fig 2B, Table S4). We followed up on this finding by comparing

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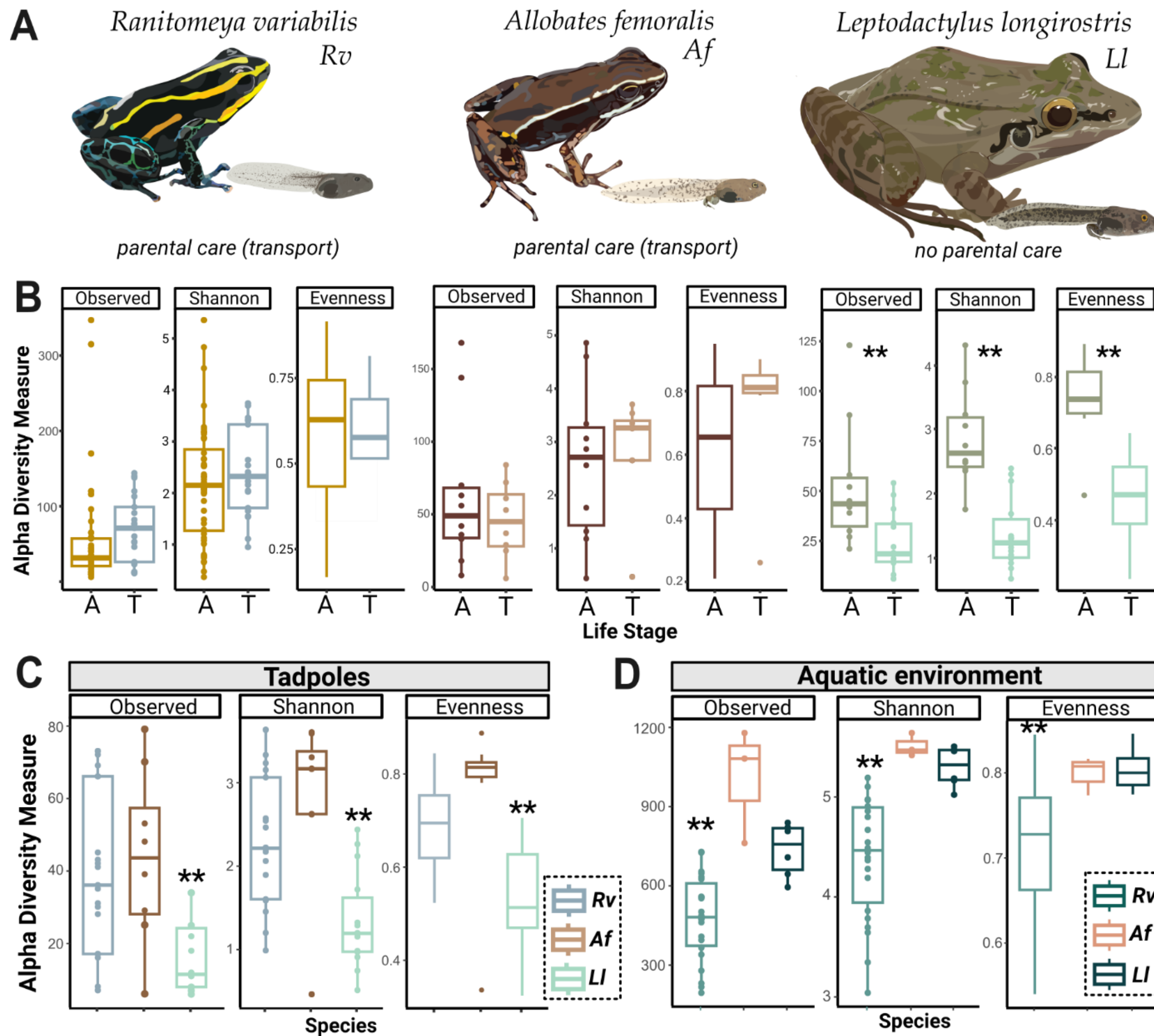
244 the diversity of tadpole-associated communities across species and found higher microbial diversity and
245 evenness in *Rv* and *Af* tadpoles compared to *Ll* tadpoles (Fig. 2C). Differences in community diversity of
246 tadpoles were not a reflection of habitat diversity: microbial communities associated with *Rv* aquatic
247 environments were less diverse and even compared to those in *Af* and *Ll* habitats (Fig. 2D). These variations
248 may therefore be linked to differing reproductive traits: *Af* and *Rv* lay terrestrial egg clutches and transport
249 hatchlings to water, whereas *Ll*, a non-transporting species, lays eggs directly in water.



250

251 **Figure 2. Tadpoles skin microbiome is shaped by their environment and is more diverse in *Rv* and *Af* compared to *Ll***
252 **tadpoles. (A)** We compared the skin microbiome of three anuran species: two species of poison frogs inhabiting different habitats
253 that transport their offspring (*Rv* and *Af*) and a leptodactylid frog (*Ll*) that deposits its eggs in water without transporting the
254 tadpoles. **(B)** Alpha diversity measures (observed ASV richness, Shannon diversity and evenness) for tadpoles (T) and adults (A)
255 of each species were compared. Differences were determined with an ANOVA or Kruskal Wallis test, significance ($p < 0.01$) is
256 indicated by **. **(C)** Comparison of ASV richness, Shannon diversity, and evenness of communities associated with poison frog
257 tadpoles (*Af* or *Rv*) and non-poison frog species (*Ll*). **(D)** Comparison of Shannon diversity and evenness of communities associated
258 with the aquatic habitats of *Af*, *Rv*, and *Ll*. Bars in boxplots represent median values. The dataset was separately rarefied to the
259 lowest read depth of each comparison.

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260 Tadpole transport is not associated with higher degree of similarity between adult and tadpole skin
261 microbiotas.

262 To assess how microbes transmitted during parental care shape communities associated with
263 tadpoles, we tested the hypothesis that tadpoles of the transporting species *Rv* and *Af* would have
264 communities more similar to conspecific adults compared to tadpoles of the non-transporting species *Ll*.
265 We first evaluated variation in bacterial communities associated with adults, tadpoles and their
266 microenvironment by using a principal coordinate analysis (PCoA) to construct ordinations based on Bray-
267 Curtis dissimilarities to explore factors that influence skin community composition (Fig. 3A). Tadpoles of
268 all species clustered separately from adults, each other, and their aquatic environment (PERMANOVA on
269 genus level, df = 8, R^2 = 0.52909, F = 18.117, p adj = 0.001) (Fig. 3A, B, Table S5). Consistent with findings
270 in pond-spawning anurans (Kueneman et al., 2014, 2016; Prest et al., 2018), skin communities varied
271 significantly across life stages in all species (PERMANOVA on genus level: species: df = 5, R^2 = 0.29674,
272 F = 11.139, p = 0.001, life stage: df = 2, R^2 = 0.23148, F = 20.331, p = 0.001). Parental care also explained
273 some of the observed variance (PERMANOVA on genus level: df = 2, R^2 = 0.07513, F = 5.4831, p = 0.001).
274 To further examine patterns of similarities between life stages, we compared the core communities of adults,
275 tadpoles and their aquatic environments across a range of prevalence and abundance cutoffs (prevalence
276 100% or 75%; relative abundance no cutoff, ≥ 0.1% (low) and ≥ 1% (high) (Table S6). Independent of
277 parenting strategy and species, tadpoles shared core communities of their microbiome with their
278 environments, but not with adult individuals. Adult poison frogs never shared core taxa with the aquatic
279 environment of their tadpoles (Fig. 3C, Table S6), even though adults were commonly found in the
280 nurseries. In contrast, the aquatic environment contributed substantially to the skin community of adult *Ll*,
281 which shared high- and low-abundance (≥ 0.1%) core genera with the pond water, but not with the pond-
282 inhabiting tadpoles (Fig. 3C, Table S7).

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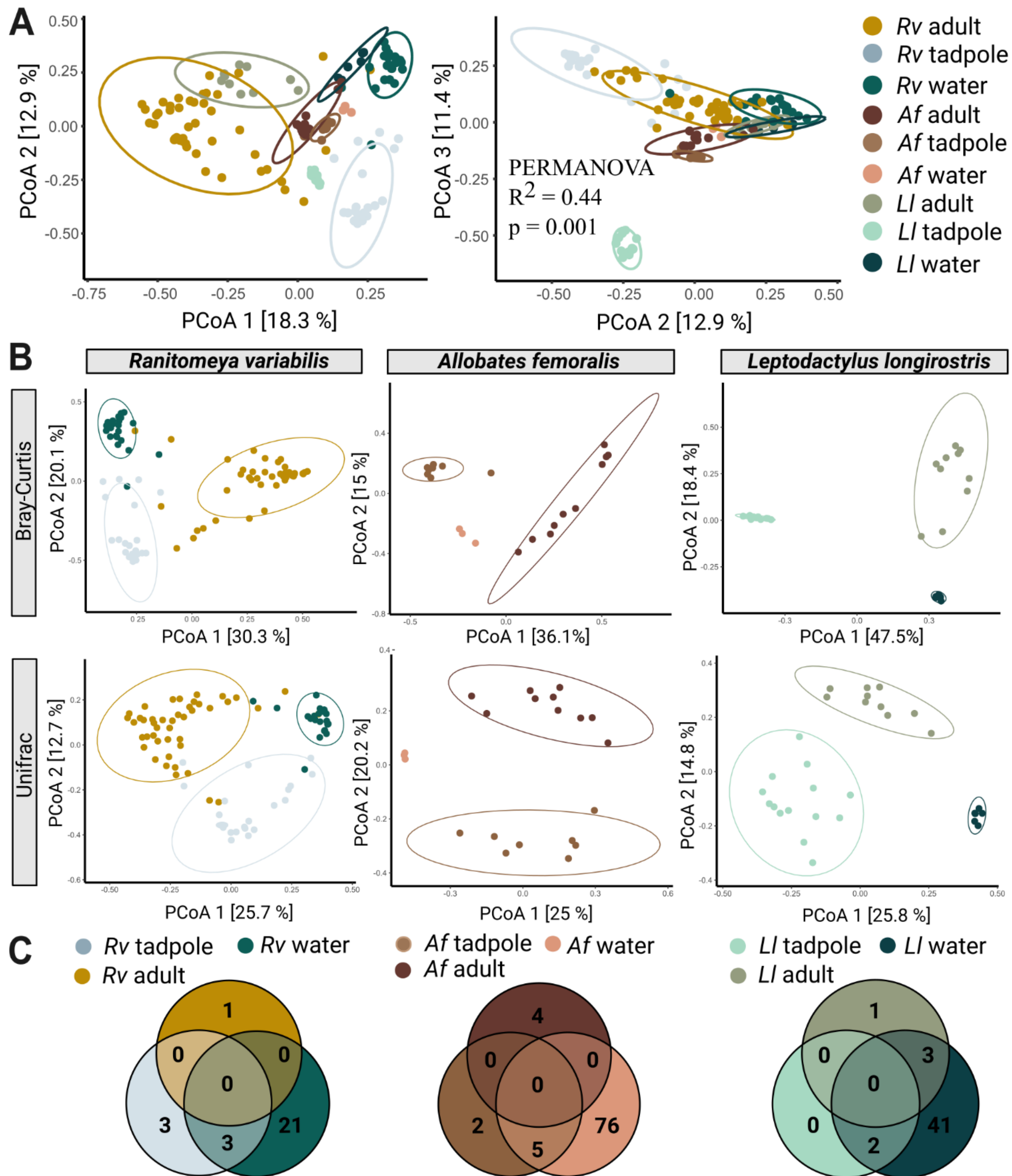

283

284 **Figure 3. Species, life stage and parental care affect clustering of microbial communities**. **(A)** In a Principal Coordinate
285 Analysis constructed with Bray-Curtis distances (axis 1 and 2 on the left, axes 2 and 3 on the right) tadpoles cluster significantly
286 differently from each other, adults, and their aquatic environment. Significances were determined with a PERMANOVA followed
287 by a pairwise adonis posthoc test. Points in ordination plots represent the communities of each sample, circles represent confidence
288 ellipses. **(B)** Principal Coordinate Analysis constructed with Bray-Curtis and Unifrac distances for adults, tadpoles, and aquatic
289 environment of each species. **(C)** Number of core species (prevalence > 75%, relative abundance > 0.1%) shared between adults,
290 tadpoles and the respective aquatic environment of each species.

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291 To assess if microbes transmitted during tadpole transport constitute a stable part of skin
292 communities associated with tadpoles, we compared community distances between skin communities of
293 tadpoles and adults of the three frog species. We predicted that tadpoles and adults might have more similar
294 skin microbiomes in the transporting species *Rv* and *Af* than in the non-transporting species *Ll*. We found
295 no difference in community distances between tadpoles and adults in *Af* and *Ll* (iterated beta regression
296 averaged over 999 random subsamples of similar sample size (IBR): Unifrac: $p = 0.3$). Communities
297 associated with *Rv* tadpoles were more dissimilar from adult communities than microbiota of *Ll* tadpoles
298 and adults (IBR: Unifrac: $p = 0.008$). Taken together, our results indicate that microbes acquired during
299 tadpole transport do not create lasting similarities in composition between tadpoles and adults of wild
300 populations.

301 <u>After 4 weeks, transported *Ranitomeya variabilis* tadpoles retain isolated members of their caretaker's core</u>
302 <u>community.</u>

303 To test how offspring transport in *Rv* affected the community composition in tadpoles, we
304 conducted a field experiment where we reared transported (N=10) and non-transported (N=10) tadpoles in
305 artificial cups. Transported tadpoles were collected from the backs of their caregiver (N= 8), while we
306 bypassed parental care in the non-transported group by transferring eggs to cups before the tadpoles hatched
307 (Fig. 4A). Two out of ten non-transported tadpoles died before sampling. Richness and evenness of tadpole
308 communities did not differ significantly between experimental conditions (Anova: Observed ASVs: df =
309 1, F = 3.1981, p.adj = 0.2780; Shannon: df = 1, F = 1.1322, p.adj = 0.9093; evenness: df = 1, F = 0.0268,
310 p.adj = 2.6163), though PCoA on Bray Curtis distances revealed differences in microbiome composition
311 attributable to the presence or absence of transport (Fig. 3B) (PERMANOVA: df = 3, $R^2 = 0.44$, F = 10.402,
312 $p = 0.001$). Compared to transported tadpoles, non-transported tadpoles showed less overlap of genera in
313 their microbial community with their aquatic environment (Fig. 3C). Specifically, the genera *Pelomonas,*
314 *Rhodomicrobium* (Proteobacteria), *Mycobacterium* (Actinobacteriota) and *Candidatus Koribacter*
315 (Acidobacteriota) primarily colonized transported tadpoles, while the genus *Limnohabitans*
316 (Proteobacteria) was found on non-transported tadpoles. *Cetobacterium* and *Burkholderia-Caballeronia-*
317 *Paraburkholderia* were found to colonize tadpoles from both experimental conditions, but at different
318 abundances (ANCOMBC 2, $p < 0.05$) (Table S8).

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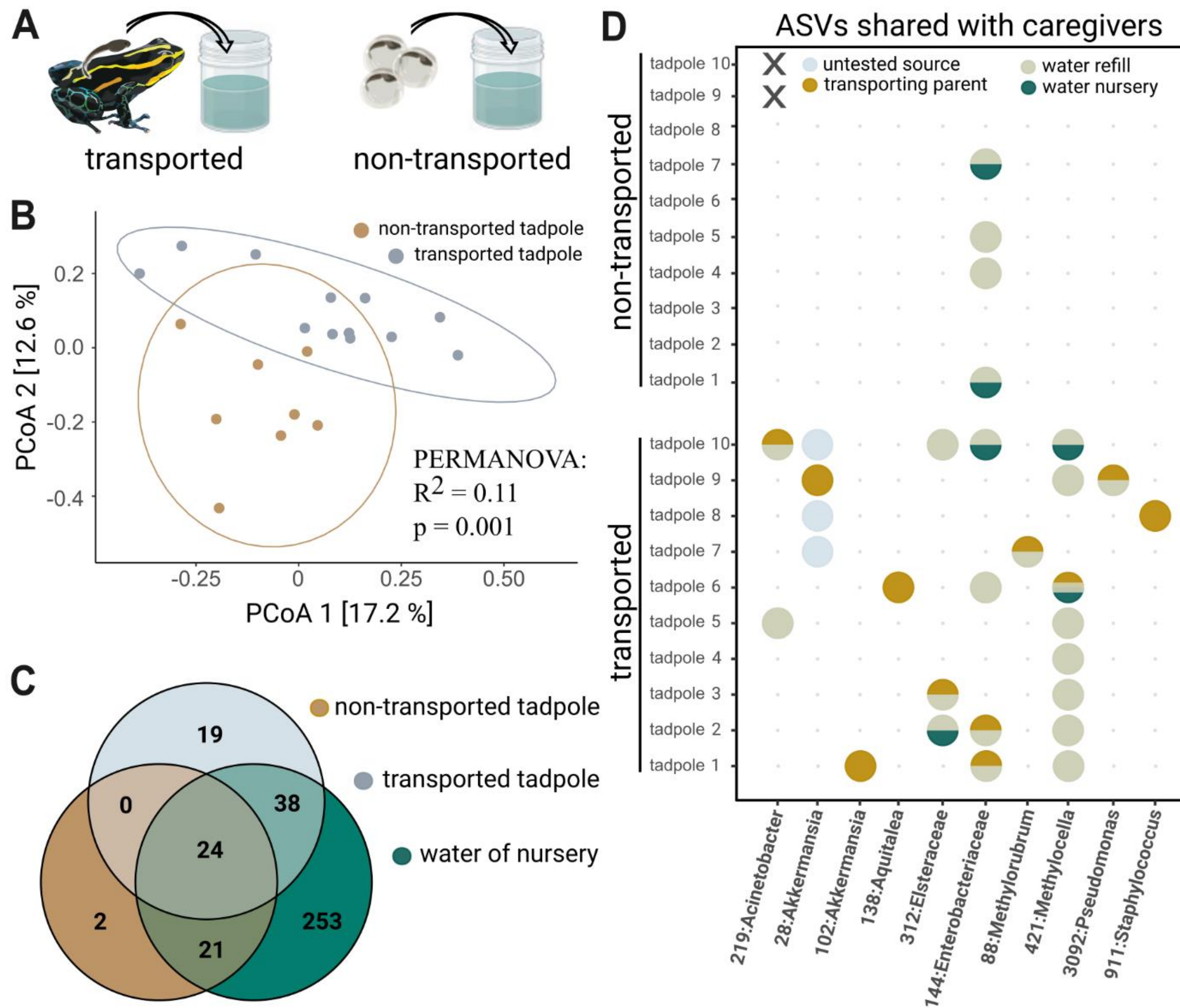

319

320 **Figure 4. Tadpole transport influences community structure. (A)** Tadpoles collected from the back of their caregiver ("transported") and reared in artificial cups for one month were compared to six-week-old tadpoles that hatched from eggs transferred to artificial cups and did not experience transport by adult frogs ("non-transported"). **(B)** Principal Coordinate Analysis constructed using unweighted Unifrac distances, transported tadpoles cluster significantly differently from non-transported tadpoles. Significances were determined with a PERMANOVA followed by a pairwise adonis post hoc test. **(C)** Venn diagram comparing unrarefied ASVs agglomerated on a genus level between transported tadpoles, non-transported tadpoles, and the aquatic environment. **(D)** Bubble diagram depicting the presence (circle) or absence (dot) of 10 ASV found to be shared between parents and transported tadpoles as well as their possible source ("transporting parent ", "nursery water", "refill water" or "untested"). Non-transported tadpoles that died prior to sampling are indicated by 'X'.
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329 We further tested the hypothesis that related tadpole-adult pairs with a history of direct contact
330 would have more similar communities than unrelated tadpole-adult pairs without such history. One month
331 after transport, we found that skin communities of tadpoles were not more similar to the transporting parent
332 than to unrelated adults of the population (iterated beta regression averaged over 999 random subsamples
333 of similar sample size (N=10) (IBR): Unifrac: p = 0.27), confirming our results from the population
334 sampling. However, a Sourcetracker analysis on unrarefied data revealed notable relative contributions of
335 the parental skin community to the community on four transported tadpoles (tadpoles 1, 2, 6, 8) (min = 0%
336 to max = 17%, median = 0%, IQR = 10.5) (Table S9) after one month of growth.

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337 We followed up on this finding by directly identifying taxa shared between caregivers and the
338 respective transported offspring without imposing an abundance cutoff. We found that 8 out of 10 tadpoles
339 shared one or two ASVs with the frog that had transported them in relative abundances ranging from 0.02
340 to 19.3 % (Fig. 4D) (Table S9). Shared taxa belong to the genera *Akkermansia, Elsteraceae, Aquitalea,*
341 *Methylocella, Methylobacterium-Methylorubrum, Staphylococcus, Pseudomonas* and *Acinetobacter*.
342 Seven out of ten shared microbes constituted members of the ten most abundant ASVs found on the
343 respective transporting frog (Table S9). A genus-level network constructed from 44 adult frogs shows that
344 the retained genera make up a central part of the community of adult *Rv* in wild populations (Fig. S5).
345 Finally, we determined the success of these ASVs in colonizing transported and non-transported
346 experimental tadpoles. Even though at least six (min: 6, avg: 6.38, max: 7) of these ten ASVs were
347 consistently present in the aquatic environment of non-transported experimental tadpoles, only one (family
348 *Enterobacteriaceae*) successfully colonized their skin (Fig 4D, not transported tadpoles) (Table S10).

349 # Discussion

350 In this study we aimed to understand the main factors influencing establishment of microbial
351 communities associated with anuran tadpoles receiving parental care. To better understand sources
352 contributing pioneering taxa, we first aimed to determine the ontogenetic window in which anuran embryos
353 are colonized. Next, we tested if a specialized anuran parenting strategy, tadpole transport, transmits early
354 colonizers. Then, we sampled natural populations of frogs to investigate if parental signatures persist into
355 later ontogenetic stages in an ecologically relevant context. We found that substantial microbial
356 colonization of the poison frog *Rv* occurs after embryos hatch from the vitelline envelope. Furthermore, we
357 demonstrated through cross-fostering experiments that transporting frogs serve as a source of skin microbes
358 for tadpoles. Next, we sampled sympatric species in a Neotropical habitat and revealed that tadpoles of
359 poison frogs showed more diverse microbial communities than tadpoles of leptodactylid frogs. Finally, a
360 field experiment in the wild population indicated that microbial communities of *Rv* tadpoles after four
361 weeks of growth were not more similar to transporting caregivers than to unrelated adults, although some
362 tadpoles did maintain isolated ASV that were shared with the transporting adults. Together, our results
363 demonstrate that microbial colonization of a poison frog tadpole begins at an ontogenetic stage immediately
364 preceding parental contact, that parental care in poison frogs facilitates the transfer of microbes to newly
365 hatched tadpoles, and that signatures of these parentally transmitted microbes can persist, albeit at low
366 prevalences, through the first month of tadpole development. This study shows that tadpole transport may
367 serve as a mechanism to transmit host-adapted microbes, thereby filling a knowledge gap in our
368 understanding of the function of parental care in anurans.

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369 Early microbial colonizers have been shown to shape community structure of newborns in insects
370 (E. W. Jones et al., 2022), mammals (e.g. Blyton et al., 2022; Kaur et al., 2022; Sprockett et al., 2018), fish
371 (e.g. Almany, 2004), birds (e.g. Chen et al., 2020) and anurans (e.g. K. R. Jones et al., 2023, 2024) via
372 priority effects (see Debray et al., 2022; Fukami, 2015 for reviews). Thus, determining critical
373 developmental periods for microbial colonization of host tissue is crucial to assess which sources contribute
374 these pioneering taxa. We detected microbes in embryos of all stages but found the abundance to be
375 considerably lower than in the surrounding jelly. Additionally, we found that substantial colonization of *Rv*
376 tadpoles happens after hatching from the vitelline membrane, at an ontogenetic stage immediately
377 preceding skin-to-skin contact with the caregiver. These results underline that the opportunistic colonization
378 of the anuran embryo is much more restricted than that of the surrounding jelly (Hayes et al., 2009;
379 Kueneman et al., 2014; Walke et al., 2011), which harbors stable microbial assemblages (Hughey et al.,
380 2017; Walke et al., 2011). Such restrictions might result from host-led selection as observed in marine
381 mammals (Switzer et al., 2023) and/or a barrier function of the vitelline membrane that limits microbial
382 access to the developing embryo. For example, the latter was described for the avian vitelline membrane,
383 where it is mediated by antimicrobial proteins (Guyot et al., 2016; Mann, 2008; Mine & Kovacs-Nolan,
384 2006). The substantial microbial colonization that occurred after hatching suggests that the anuran vitelline
385 envelope plays an important role in restricting microbial access to poison frog embryos until they are
386 sufficiently developed to be transported by a caregiver. Those bacteria that are present in unhatched tadpoles
387 might have been acquired prior to hatching, such as in the case of the amphibian symbiont *Oophila*
388 *amblystomatis* (Kerney et al., 2011; Kim et al., 2014), which is acquired by oviductal transmission and
389 grows on the inside of individual salamander and frog vitelline membranes. Alternatively, the vitelline
390 membrane may function as a selective barrier, permitting the passage of certain taxa, with hosts acting as
391 additional filter to select suitable colonizers. Future studies should therefore address the presence of
392 antimicrobial proteins, biofilms, and immune components in the anuran embryo and vitelline membrane to
393 shed light on the mechanisms that regulate microbial colonization of anuran embryos.

394 Across species, newborns might acquire bacteria not only through horizontal transfer from
395 environmental source pools (e.g. arthropods: Douglas, 2018; Mushegian et al., 2018, amphibians: Rebollar
396 et al., 2016, mammals: Bik et al., 2016) but also through vertical transmission during parent-offspring
397 interactions (reptiles: Bunker et al., 2021; fish: Sylvain & Derome, 2017; humans: Wang et al., 2020).
398 Modes of parental inheritance involve indirect transmission, like contact smearing onto the egg surface
399 during or after oviposition, as found in European firebugs (Salem et al., 2015), and direct social acquisition,
400 as observed in social bees (Kwong et al., 2017; Leftwich et al., 2020). Previous studies in anurans have
401 investigated vertical transmission of microbes during parental care in the context of indirect transmission

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402 during egg attendance without considering less widespread parenting strategies involving direct parental
403 contact. These studies did not find evidence for parental inoculation, as hatched tadpoles supported bacterial
404 communities very similar to adult frogs but independent of caregiver identity (Hughey et al., 2017). In
405 contrast, we investigated vertical transmission in an anuran system where parenting involves direct skin-
406 to-skin contact between tadpoles and their caregiver. We found that both transported and non-transported
407 tadpoles in our cross-foster experiment shared microbial ASVs with each other, indicative of acquisition of
408 microbes from the jelly upon hatching as demonstrated for other anuran clades (Hughey et al., 2017; K. R.
409 Jones et al., 2024; Warne et al., 2017). After six hours of contact, transported tadpoles -but not their siblings-
410 shared more microbes with the surrogate heterospecific frog than with the parental species, suggesting that
411 microbes can transmit vertically during parental care in poison frogs. Thus, in this clade both clutch
412 environment and transporting caregivers can serve as source pool for hatched tadpoles.

413 Variation in the source proportions that transported tadpoles acquired from transporting frogs
414 suggests that additional factors modulate the efficacy of vertical transmission in poison frogs. Evidence for
415 duration-dependent microbial transmission is seen in humans, where recent research highlights a
416 cumulative contribution of the paternal microbiome to the assembly of infants' microbial communities
417 (Dubois et al., 2024). In anurans, the time that hatched tadpoles spend in contact with jelly-associated
418 microbes or in skin-to-skin contact with parents might therefore affect the success and stochasticity of
419 colonizers that are transmitted during parental care. These transport logistics vary between and within
420 anuran species (Altig & McDiarmid, 2007; McDiarmid & Altig, 1999) and are closely linked to trade-off
421 decisions of the caregivers, which often need to balance territory defense, mating, and caring for offspring
422 (Pašukonis et al., 2019; Ringler et al., 2013; Wells, 2007). For instance, *Rv* males often assist tadpoles with
423 hatching by tearing the capsule apart with their rear legs to initiate transport (Brown et al., 2008), but have
424 also been observed to pick-up free-swimming tadpoles (Schulte & Mayer, 2017). Likewise, *Af* tadpoles
425 have been observed to remain in the jelly for several days before being transported (Peignier et al., 2022;
426 Ringler et al., 2013). Parentally transmitted microbes might thus constitute pioneering taxa in an unoccupied
427 niche if caregivers assist with hatching or encounter established assemblages as in the latter scenario.
428 Prolonged parental care has been shown to enhance the generational transmission of symbiotic
429 microorganisms in burying beetles (e.g. Körner et al., 2023) and thus, duration of the skin-to-skin contact
430 during tadpoles transport might influence microbial acquisition in a time-dependent manner. How transport
431 durations which range from a few hours to several days depending on the species (Lötters et al., 2007;
432 McDiarmid & Altig, 1999; Pašukonis et al., 2019) affect stochasticity of transmitted microbes remains
433 unexplored. Collectively, our findings suggest that early life microbes are introduced to the poison frog
434 host in a mixed transmission mode, where community members can be sourced environmentally from the

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435 egg jelly and socially from a caregiving frog. Recent evidence from Kouete et al. documented the first case
436 of vertical transmission in amphibians, observed in subterranean caecilians that protect and feed their
437 offspring (Kouete et al., 2023). To our knowledge, this is the first study that extends evidence for vertical
438 transmission during parental care to the order of anurans.

439 To investigate if parental signatures persist into later ontogenetic stages, we tested the hypothesis
440 that a history of direct contact would result in more similar skin communities between tadpoles and
441 caregivers relative to unrelated adults. Based on previous findings which documented that priority
442 inoculation of tadpoles increased the relative abundance of some beneficial inoculates (K. R. Jones et al.,
443 2024), we hypothesized that vertical transmission manifests in persistent associations with parentally
444 acquired microbes. Similar to Hughey et al., we found that vertical transmission did not lead to a higher
445 degree of similarity between caregiver and tadpole skin microbiota relative to unrelated adult-tadpole pairs.
446 Likewise, communities associated with adults and tadpoles of transporting species were no more similar
447 than those of non-transporting species. While tadpoles do acquire caregiver-specific microbes during
448 transport, these results suggest that most of these microbes do not persist on the tadpoles' skin long-term.
449 A similar pattern of convergence in the composition of tadpole-associated communities has been observed
450 previously in wood frogs inoculated with gut microbes from bullfrogs (Warne et al., 2019). Interestingly,
451 as documented in fruit flies (Cox et al., 2014), effects of early microbial disruption on physiology and
452 disease susceptibility of the anuran host persisted even after community structures converged (Warne et al.,
453 2019). Therefore, it is possible that vertically acquired microbes shape communities of poison frogs in
454 different ways than by gaining abundance priority on tadpole skin. For example, they may durably imprint
455 the immune system (Fallet et al., 2022; Gensollen et al., 2016) and alter host metabolic phenotypes (Cox et
456 al., 2014; Sommer & Bäckhed, 2013). Additionally, isolated ASVs contributing to central genera of
457 communities on adults persisted on some tadpoles in varying abundance. There is clear evidence that low-
458 abundant keystone taxa, despite their rarity, can act as drivers of compositional changes (Han & Vaishnava,
459 2023). For instance, low abundance ASVs drive compositional changes in the hindgut of subterranean
460 termites after dietary alterations (Benjamino et al., 2018) and confer resistance to Salmonella-induced
461 colitis in mice (Herp et al., 2019). Moreover, despite their abundance in the water of all tadpole nurseries,
462 most of the ASVs that tadpoles shared with caregivers failed to colonize non-transported hosts. These
463 results suggest that vertical transmission may be a more effective mode of transmission of host-adapted
464 microbes compared to horizontal transmission from the environment. Whether vertically transmitted
465 microbes shape the anuran immune response and if retaining certain ASVs allows them to become dominant
466 community members after metamorphosis remains to be investigated.

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467 We found that the higher diversity of communities associated with tadpoles of species that exhibit
468 offspring transport was not linked to the diversity found in tadpoles' aquatic habitat but might instead be
469 associated with other life history traits. Although skin alkaloids are found on many adult poison frogs
470 including *Rv* and influence skin community composition (Caty, Alvarez-Buylla, et al., 2024), they are not
471 present on adult *Af* or on tadpoles of any species included in this study (Villanueva et al., 2022) and are
472 thus unlikely to cause the observed differences in diversity. It is possible that oviposition strategy or
473 physiological characteristics like skin pH or abundance of antimicrobial proteins (Conlon, 2011; Faszewski
474 et al., 2008; Kueneman et al., 2014) contributed to increased diversity in *Rv* and *Af* relative to *Ll* tadpoles.
475 Alternatively, transport might have been co-opted in an evolutionary context to increase microbial diversity
476 in tadpoles and shape immune recognition and health in the anuran host in a manner comparable to humans
477 (Al Nabhani & Eberl, 2020; Donald & Finlay, 2023; Gensollen et al., 2016). Increased microbial diversity
478 is linked to better health outcomes, including improved immune function, lower disease risk, and enhanced
479 overall fitness in other vertebrate classes. For example, higher diversity of gut microbial communities is
480 associated with enhanced exploratory behavior in songbirds (Florkowski & Yorzinski, 2023) and improved
481 pathogen resilience in fish (de Bruijn et al., 2018). Previous research in frogs has linked higher diversity of
482 tadpole skin communities to enhanced parasite resistance later in life (Knutie et al., 2017; Warne et al.,
483 2019). Further studies across more transporting and non-transporting species are needed to assess the role
484 of transport in shaping diversity of tadpole-associated communities. Moreover, the low chytrid infection-
485 rate in the studied natural populations limits the ability to draw conclusions about differences in disease
486 susceptibility within and between species or life stages in our dataset.

487 Elucidating the key factors that select for parental care and account for the diversity of care
488 strategies across various species remains a challenge in the field of evolutionary and behavioral ecology
489 (Balshine, 2012; Clutton-Brock & Scott, 1991; Gross, 2005; Royle et al., 2012). Microbiota management
490 is documented to actively drive, rather than merely accompany sociality and parenting behavior in other
491 organisms like carrion feeding insects (Biedermann & Rohlfs, 2017; Körner et al., 2023) and has recently
492 been proposed as a factor driving the evolution of paternal care in vertebrates (Gurevich, 2020). Even
493 though commensal skin microbiota undoubtably play a pivotal role in the health and pathogen defense of
494 amphibians, it remains poorly understood how complex parental care strategies in this class aid microbial
495 transmission. This is especially surprising as amphibians have recently been identified as the most
496 threatened class of vertebrates (Daszak et al., 2003; Luedtke et al., 2023; Wake & Vredenburg, 2008) due
497 to their susceptibility to globalization-related threats like climate change and disease-spread. Our
498 observation that anuran parental care transmits microbes fills a knowledge gap about the function of

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499 parental care in this class and highlights the need for further studies across more anuran species with
500 contrasting reproductive strategies.

501 # Methods

502 Captive-bred animals

503 Captive-bred *Rv* of our laboratory colony are kept non-sterile and reproduce year-long. Males attend the
504 terrestrial clutch of 3-5 eggs and transport larva to water when they reach Gosner stage 24-25 where they
505 hatch from the vitelline membrane. *Rv* were housed in pairs and provided with horizontally mounted film
506 canisters as egg deposition sites, and film canisters filled with water (treated with reverse osmosis R/O Rx,
507 Josh's Frogs, Owosso, MI) for tadpole deposition (Goolsby et al., 2023).

508 Microbial colonization of embryos and egg jelly

509 We used individually packed sterile transfer pipets (Samco™, Thermo Scientific, 7.7 mL, cat # 202-1SPK)
510 with a cut tip to move eggs and embryos. All tools were treated with 8.25% Sodium Hypochlorite
511 (CloroxPro) and wiped down with 70% Ethanol before use and between tissues and tadpoles. The
512 experimenter always wore gloves that were changed between individuals and disinfected with 70% Ethanol
513 before handling frogs.

514 Triplicates of five different developmental stages (Gosner, 1960) before (stages 10, 19, 23, 24) and after
515 hatching from the vitelline membrane (stages 24, 25) were collected from 3 different breeding pairs. We
516 manually separated the jelly from the egg sac with autoclaved forceps, a procedure commonly done in
517 preparation for microinjections. The embryo in the vitelline membrane was transferred to a small petri dish
518 with sterile, filtered deionized water and opened using forceps. Tadpoles were transferred to a new petri
519 dish with clean water twice to remove transient bacteria from the skin and were then euthanized and
520 homogenized. Organic matter was removed from the jelly, and both jelly and tadpole tissues were flash
521 frozen and stored at -80 °C until DNA isolation. Isolation of DNA was performed as detailed below, and 1
522 µL of isolated DNA was used for PCR amplification of the full length 16S rRNA region (~1500 bp) with
523 1 µL of 10 µM forward 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and reverse primer 1492R (5'-
524 TACGGYTACCTTGTTAYGACTT-3') (Fredriksson et al., 2013), 12.5 µL of OneTaq Hot Start Quick
525 Load polymerase (New England Biolabs) and 9 µL water. We followed an amplification protocol with
526 denaturation for 30 s at 94 °C followed by 30 cycles of denaturation for 15 s at 94 °C, annealing for 30 s at
527 58 °C and extension for 2 min at 68 °C and a final extension period of 5 min at 68 °C. To determine the

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528 presence or absence of amplification, we visualized 5 μL of each PCR product on a 1% Agarose gel (90mV
529 for 90 min) with 2 μL of 1kb GeneRuler for sizing.

530 *Quantitative analysis of 16S rRNA copy numbers with droplet digital PCR (ddPCR)*

531 Quantification of prokaryotic concentration for each sample was determined by ddPCR for all samples.
532 Sample DNA were serially diluted 1:10 in sterile nuclease-free water to dilute the concentration of any PCR
533 inhibitors using a liquid handler (Agilent, Agilent Velocity 11 Vprep). 16S rRNA concentrations from
534 embryonic tissue are reported from undiluted DNA as its concentration was approaching the limit of
535 detection in diluted reactions. Universal 16S rRNA primers (331F/797R) and 16S rRNA HPLC-purified
536 FAM probes were used, as previously described (Langenfeld et al., 2021). Primer-probe mixture was
537 created by mixing forward primer, reverse primer, and probe 1:1:1 for a total volume of 0.264uL per
538 reaction. Each ddPCR reaction was composed of 11uL of ddPCR Supermix for probes (Bio-Rad, 1863024),
539 0.264uL of primer-probe mixture, 4.736uL of sterile nuclease-free water and 6uL of sample, for a total of
540 22uL/reaction. As a positive control, equal amounts of NIST Microbial Pathogen DNA Standards for
541 Detection and Identification (NIST, RM8376) components A through R were combined in equal amounts.
542 Each ddPCR plate included a positive control, NIST mixture, and negative control, sterile nuclease-free
543 water, in quadruplicate. ddPCR reactions were performed with the QX200 AutoDG Droplet Digital PCR
544 system (Bio-Rad). PCR amplification was performed with the Bio-Rad T100 thermocycler using the
545 following program: 95°C for 10 minutes, 40 cycles of 95°C for 30 seconds and 56°C for 1 minute and 72°C
546 for 2 minutes, followed by 1 cycle of 4°C for 5 minutes and 95°C for 5 minutes with a ramp speed of
547 2°C/second at each step. Amplified reactions were quantified using a ddPCR reader. Thresholds were set
548 for each ddPCR reaction based on the negative and positive control rain plots generated by the QX Manager
549 Software, 2.1 Standard Edition (Bio-Rad). All reactions had greater than 15,000 accepted droplets. 16S
550 rRNA copies per droplet were calculated by taking the natural logarithm of the ratio of accepted droplets
551 to negative droplets for each reaction. Values were corrected for droplet volume and ddPCR reaction
552 volume to calculate total 16S rRNA copies per reaction. We further calculate and report the total 16S rRNA
553 copies per μL by accounting for the sample ddPCR volume, dilution factor, and DNA extraction volume.
554 Differences in copy number concentrations detected in jellies, hatched and unhatched tadpoles were
555 determined using a Kruskal Wallis test with p-values adjusted for multiple comparisons using Benjamini
556 Hochberg correction (R Core Team, 2023) and followed by a Dunn test.

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557 Vertical transmission of bacteria

558 Siblings of one clutch were randomly assigned to one of three treatments: (1) no transport, (2) transport by
559 the biological caregiver, or (3) transport by a heterospecific surrogate frog. We chose the poison frog
560 *Oophaga sylvatica* as foster frogs because adults are docile, parental care in this species involves
561 transporting tadpoles, and skin microbial communities differ from *Rv*. Tanks of *Rv* breeding pairs were
562 monitored for clutches of at least three eggs daily. Embryos assigned to group one or three were placed into
563 a petri dish in a separate tank without access to adults when tadpoles reached a developmental stage close
564 to hatching (Gosner stage 22) to bypass transport by the biological father. The remaining eggs in the parental
565 tank were monitored with security cameras (Wyze v3, Goolsby et al., 2023) that notify the user of any
566 movement in the canister. After tadpole pickup by the biological father, all water canisters for tadpole
567 deposition were removed from the tank to standardize transport time to 6 hours for both groups. Siblings
568 of group three were transferred to a new petri dish, hatched using a sterile brush and directly transferred to
569 the back of a surrogate frog (as described in Pašukonis et al., 2017) that was caught in a fresh plastic bag
570 and rinsed with sterile water. The surrogate frog was placed into a plastic containment (Sterilite 16428012)
571 with moist, autoclaved paper towels for the duration of the experiment. After 6 hours, the transporting
572 biological father and the surrogate frog were caught in a fresh plastic bag. Each tadpole was removed from
573 the back and washed twice to remove transient bacteria by transferring them to new petri dishes with 10 ml
574 sterile water. Non-transported tadpoles were removed from the jelly and washed in a similar way. Each frog
575 was rinsed with sterile water, and a skin swab of their back was collected using a sterile Puritan applicator
576 (Puritan Medical Products, 25-206 1PD BT) (Caty, Alvarez-Buylla, et al., 2024; Caty, Vasek, et al., 2024).
577 All tadpoles were euthanized, their skin was collected, and skin and applicators were stored at -20 °C until
578 DNA extraction. We chose to sample whole skin instead of swabs to detect small amounts of transferred
579 bacteria on and in the skin.

580 Field site and natural populations

581 The natural study site of *Rv* and *Leptodactylus longirostris* (*Ll*) is situated on top of the mountain 'Inselberg'
582 in vicinity to the Centre Nationale de la Recherche Scientifique managed research station (4°5' N, 52°41'
583 W) within the Nature Reserve ´Les Nouragues´ in French Guiana (Bongers et al., 2011). Patches of Clusia
584 trees are separated by bare granite rocks and exposed to extreme environmental conditions (e.g. temperature
585 oscillations between 18°C - 75°C (Sarthou, 2001). *Rv* and *Ll* (IUCN Conservation status: Least Concern)
586 inhabit the granite outcrop of the study site, *Af* inhabit forest floors of primary terra-firme forests
587 surrounding the campsites 'Inselberg' and 'Saut Pararé' (4°02'N/52°41'W) in the Nouragues reserve.

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588 Field study species, reproductive strategies and life history

589 *Ranitomeya variabilis (Rv)* (Zimmermann & Zimmermann 1988; CITES Appendix II, IUCN Conservation
590 status: Least Concern) is a small diurnal poison frog (Family *Dendrobatidae*) with male uniparental care
591 that lives largely arboreal and uses bromeliads as a resource for reproduction. Adult frogs defend territories,
592 are polygamous and typically lay clutches of 3-4 eggs into small arboreal bromeliads (*Catopsis*
593 *berteroniana*) that are abundant on Clusia trees in the rock savanna. Male frogs transport hatched tadpoles
594 to individual water bodies that form in the leaf axils of the large terrestrial tank bromeliads (*Aechmea*
595 *aquilega*). The cannibalistic tadpoles grow up in individual pools of ~80 ml (Poelman et al., 2013) where
596 they feed on algae and leaf debris until they complete their development after about 3 - 5 months (Poelman
597 & Dicke, 2007).

598 *Allobates femoralis (Af)* (Boulenger, 1884; CITES Appendix II, IUCN Conservation status: Least Concern)
599 are polygamous diurnal poison frogs (Family *Aromobatidae*) that inhabit the understory of primary tropical
600 forests. Females are attracted by advertisement calls of the territorial males and deposit clutches of 11-25
601 eggs in the leaf litter (Fischer et al., 2020; Stückler et al., 2019; Weygoldt, 1980). Fathers care for their
602 offspring by attending clutches and shuttle hatched tadpoles to water bodies where the social tadpoles grow
603 up sharing a pool (Lescure, 1976; Ringler et al., 2013, 2018).

604 *Leptodactylus longirostris (Ll)* (Boulenger, 1882; IUCN Conservation status: Least Concern) are medium
605 sized nocturnal leptodactylid frogs (Family *Leptodactylidae*) that inhabit the rock outcrops. They shelter in
606 rock crevices and ground covering vegetation during the day and dwell in seasonal rock pools on the
607 Inselberg plateau during nights. They breed in the rainy season and lay their eggs in ephemeral rock pools.
608 After clutch deposition, they do not care further for their tadpoles, which collectively grow up in the
609 deposition pond and feed on algae and plant debris.

610 Sampling of frogs in the field

611 Frogs were caught in fresh plastic bags upon encounter, rinsed with sterile water to remove transient
612 bacteria and soil particles and then swabbed with 20 strokes each on the dorsal and lateral sides as well as
613 on each leg and between the toes using Sterile Polyester Applicators (Puritan Medical Products, 25-206
614 1PD BT). Due to limitations in the availability of dry ice at the remote study site, applicators were directly
615 transferred into 800 µL of buffer CD1 (a lysis buffer containing chaotropic salts) of the Qiagen PowerSoil
616 Pro extraction kit and frozen until DNA extraction. Previous studies have found no difference in OTU
617 richness and evenness between native frozen and lysis buffer-stored swabs (Hallmaier-Wacker et al., 2018).

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618 Field sampling of tadpoles and their environment

619 To avoid disturbing microbial communities of tadpoles and their habitats before sampling, free-swimming
620 tadpoles were visually categorized as "small" (with a body length of less than 6 mm, typically Gosner stages
621 25 and 26), "medium" (with a body length exceeding 6 mm but no visible dorsal pattern, stages 26 - 33),
622 "large" (visible yellow dorsal pattern, stages 34 - 41) or "metamorph" (front limbs visible, stages 42 - 46).
623 Tadpoles of stages 29 – 41 (Gosner) were collected from bromeliad pools for swabbing when they at least
624 doubled the body size of freshly hatched tadpoles, at the earliest four weeks after deposition. The water and
625 sediment of the nursery was sampled prior to tadpole extraction by submerging a sterile applicator tip into
626 the leaf axil pool and moving it over all plant material contained in the water for 10 seconds. *Rv* tadpoles
627 were extracted from bromeliad leaf axils with a custom designed vacuum extractor consisting of an inlet
628 hose wide enough to allow the passage of a late-stage tadpole and a suction hose with air filter, both tightly
629 connected to the lid of a one-liter bottle. The inlet hose was submerged into the bromeliad pool while
630 applying suction to the second hose to create a vacuum that transferred the pool content into the collector.
631 After each use, all parts of the collector were cleaned with soapy water and rinsed, followed by a sodium
632 hypochlorite disinfection for 1 hour and a rinse in 70% Ethanol as described for the dissection tools. After
633 collection, tadpoles were transferred to a petri dish with sterile water twice, using individual sterile transfer
634 pipettes with a cut tip. For swabbing, tadpoles were collected in the bulb of a new sterile pipette. The water
635 was discarded, and the tadpole was swabbed on all body parts with a sterile applicator tip by moving the
636 tip over its body for 10 seconds. We further processed swabs in the same way as adult swabs. *Af* tadpoles
637 of stage 25 - 26 (Gosner) were collected from an artificial pool in the vicinity of camp 'Saut Parare'. *Ll*
638 tadpoles of Goser stage 34 - 41 were collected from three natural pools in the study plot 'Inselberg'. We
639 sampled triplicates of each pool containing multiple tadpoles. All tadpoles were processed for swabbing in
640 a similar way as *Rv* tadpoles.

641 Field experiment to compare transported and non-transported *Rv* tadpoles

642 To directly compare parental frogs with transported and non-transported tadpoles while reducing predation
643 and variation in the quality of their nurseries, we reared ten tadpoles that were collected from the back of
644 eight caregivers and ten tadpoles that hatched from eggs without experiencing transport in polypropylene
645 cups (USP #77876, 60 ml). Each cup was punctured at the 50 ml water level, covered with a sterilized
646 mosquito net secured with a rubber band to minimize predation, and attached to the stem of a Clusia tree.
647 All tadpoles received water collected from bromeliads as their initial aquatic environment with Clusia
648 leaves as shelter and food. Cups were exposed to rain and during dry periods, rainwater was collected and
649 mixed with leaf litter to refill cups. Triplicates of water samples were obtained using the procedure

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650 described for bromeliad water. We caught transporting frogs upon encounter, transferred tadpoles from
651 their backs to cups, and swabbed the parenting frog as described above. To avoid transport, eggs from 5
652 clutches were transferred to cups before tadpoles hatched. Tadpoles were sampled after growing for at least
653 26 days (min: 26, max: 77), when all tadpoles of a group exceeded ~5 mm in body size, as smaller
654 individuals exhibited increased mortality after swabbing in previous lab experiments. After swabbing,
655 tadpoles were measured and photo documented.

656 DNA extraction and 16S rRNA gene sequencing

657 The Qiagen PowerSoil Pro Kit was used to extract DNA from all swabs and tissues. The protocol was
658 adapted for use with swabs as described previously (Caty, Alvarez-Buylla, et al., 2024). DNA
659 concentrations were quantified using a Qubit. Samples were pooled by volume and the V4 region of the
660 16S rRNA gene was amplified using 515F (GTGYCAGCMGCCGCGGTAA) and 806R
661 (GGACTACNVGGGTWTCTAAT) primers (Bletz et al., 2017) and barcoded using standard Illumina
662 unique dual indices (UDIs). We performed two separate sequencing runs: Laboratory collected samples
663 were sequenced in a 2x300nt paired-end configuration on an Illumina MiSeq v3 run, field collected samples
664 in a 2x250nt paired-end configuration on a NovaSeq 6000 SP Flowcell (Roy J Carver Biotechnology
665 Center, University of Illinois).

666 Sequence processing and bioinformatics

667 We annotated in-line barcodes based on the first 7 bases of each sequencing read (umi-tools, Smith et al.,
668 2017), split out reads that matched each known barcode combination (grep, GNU Project, 1998), trimmed
669 the remaining primer sequences from the sequencing reads (cutadapt, Martin, 2011) counted the number of
670 sequencing reads in each file and removed files (including negative controls) with low read numbers (<100
671 reads) from the dataset. We processed the remaining reads with the R Divisive Amplicon Denoising
672 Algorithm package "dada2" (version 1.28.0) (Callahan et al., 2016). Taxonomy was assigned using the
673 Silva 138 database (Ref NR99) (Quast et al., 2012). The count table, taxonomy table, and sample-associated
674 data were integrated into a phyloseq object using the R package "phyloseq" (version 1.44.0) (McMurdie &
675 Holmes, 2013) for further data analysis. Any ASVs detected in control sequencing reactions without DNA
676 as well as ASVs with a phylum designation that was "NA", Eukaryotic, belonging to the family of
677 Mitochondria or the class of Chloroplasts were removed from the datasets. Samples with very low reads
678 (clearly below the first quartile) were excluded from each group.

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679 ITS sequence processing and bioinformatics

680 Amplification targeted the second internal transcribed spacer (ITS2) region of the fungal ribosomal cistron
681 using primers ITS3 and ITS4 (White et al., 1990). Full primer sequences included Illumina Nextera
682 transposase adapters (underlined) and were as follows: ITS3-5'-TCG TCG GCA GCG TCA GAT GTG
683 TAT AA GAG ACA GGC ATC GAT GAA GAA CGC AGC-3' and ITS4-5'-GTC TCG TGG GCT CGG
684 AGA TGT GTA TAA GAG ACA GTC CTC CGC TTA TTG ATA TGC-3'. Reaction mixes contained 1x
685 AccuStart II PCR SuperMix (Quantabio), 0.4 µM forward and reverse primers, and 2 µl of template DNA.
686 The thermal cycling regime consisted of an initial denaturation and enzyme activation step at 94°C for 3
687 min, followed by 30 cycles of 94°C for 45 sec, 50°C for 60 sec, and 72°C for 90 sec, with a final extension
688 step at 72° for 10 min. Ten additional PCR cycles were used to tag diluted amplicons with unique dual
689 indices (Illumina; cycling regime as above but with annealing at 54°C). Amplicons were pooled, cleaned
690 using AMPure XP beads (Beckman Coulter), and sequenced on an Illumina MiSeq instrument (2 x 300 nt)
691 at the DNA Services Lab, Roy J. Carver Biotechnology Center, University of Illinois at Urbana-Champaign.
692 Reads processing was consistent with the DADA2 ITS workflow
693 (https://benjjneb.github.io/dada2/ITS_workflow.html). Raw reads were trimmed of non-biological
694 sequence using cutadapt (Martin, 2011). DADA2 was used to filter and truncate the reads, infer amplicon
695 sequence variants (ASVs), and remove chimeras (Callahan et al., 2016). Taxonomy was assigned using
696 DADA2's implementation of the RDP naïve Bayesian classifier (Q. Wang et al., 2007) against a UNITE
697 reference database (version 9.0; Abarenkov et al., 2023). The ASV count table, taxonomy table, and sample-
698 associated data were integrated into a single object in R using the phyloseq package (McMurdie & Holmes,
699 2013).

700 Microbiota composition analyses

701 All statistical analyses were performed in R (v 4.3.2) (R Core Team, 2023), and "ggplot2" (v 3.4.4)
702 (Wickham, 2016) was used for visualizations if not stated otherwise. Illustrations of all species were created
703 in Adobe Illustrator (v 28.3) (Adobe Inc., 2024) and all figures were compiled in InkScape (v 1.1) (Inkscape
704 Project, 2021).

705 *Microbiota composition analyses of captive frogs and source tracking*

706 First, we calculated beta diversity between skin communities of adult frogs that served as transporting frogs
707 to determine if communities are distinct enough to distinguish them as microbial source communities. We
708 applied Principal Coordinates Analysis (PCoA) using the package "phyloseq" to visualize variation in the
709 genus-agglomerated dataset based on two dissimilarity indices: Bray-Curtis, a widely used measure
710 considering relative abundances and unweighted Unifrac, a presence-absence measure of ASVs based on

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711 phylogenetic distances. Data were converted to relative abundances before calculating Bray-Curtis or
712 rarefied using a rarefaction over 300 iterations (function phyloseq_mult_raref_avg in the package
713 'metagmisc' (Mikryukov, 2023) before calculating Unifrac distances. We confirmed equal dispersion
714 between compared groups using the betadisper function of the package "vegan" (v2.6.4) (Oksanen et al.,
715 2022) followed by a Tukey post-hoc test. Variation was analyzed with a PERMANOVA (function 'adonis'
716 within the package "vegan") using Bray-Curtis and unweighted Unifrac dissimilarities as responses and
717 species or transporting species as predictors. Significant PERMANOVAs were followed by pairwise
718 multilevel post-hoc comparisons between groups with the function pairwise.adonis (package
719 "pairwiseAdonis", (Arbizu, 2017). We applied the same method to compare if communities between
720 tadpoles that had been transported by a homospecific frog (*Rv*) or a heterospecific frog (*Os*) differed after
721 six hours of tadpole transport. We further used 'Sourcetracker' (Knights et al., 2011) to determine whether
722 transporting frogs served as a source for bacteria detected on tadpole skin after six hours of transport. This
723 Bayesian approach was developed to estimate proportion of contaminants in a given community that come
724 from possible source environments and is commonly used to determine if parental communities serve as
725 source for offspring communities (Kouete et al., 2023; Murphy et al., 2023; Switzer et al., 2023). We
726 performed two sets of analysis to determine the source proportions of adult *Rv* and *Os* in tadpole
727 communities. First, we defined transporting frogs of both species as possible microbial sources and all
728 tadpoles including non-transported controls as sinks. Then, we defined transporting frogs and non-
729 transported control tadpoles as possible sources to visualize the proportion of bacteria acquired in the clutch
730 prior to transport. For both approaches, we rarefied the source dataset of transporting frogs to decrease the
731 influence of high-coverage source samples on the analysis. For the first approach, differences in community
732 proportions shared with the transporting species ('transporting frog') or the non-transporting species ('non-
733 transporting frog') were determined for non-transported and transported tadpoles and compared using a
734 Kruskal Wallis test, with p-values adjusted for multiple comparisons using Benjamini Hochberg correction
735 (R Core Team, 2023). Additionally, we plotted the source proportion of *Rv* and *Os* communities for non-
736 transported tadpoles, tadpoles transported by *Rv*, and tadpoles transported by *Os*, and we performed the
737 same analysis between the experimental conditions for each source (*Rv* or *Os*) separately. For the second
738 approach, we determined the source proportions that transported tadpoles had acquired from the clutch,
739 from *Rv* adults, and from *Os* adults, and we determined differences between tadpoles transported by *Rv* or
740 by *Os* using a paired Wilcoxon test with Benjamini Hochberg correction (R Core Team, 2023).

741 *Microbiota composition analyses of wild populations*

742 We calculated mean and standard deviation across all samples per group for the number of phyla and
743 families from a rarefied dataset to compare groups. The total number of unique phyla and families for each

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744 group was calculated from the unrarefied dataset to include low abundance taxa. We plotted the relative
745 abundance of taxa agglomerated at the phylum level with an abundance greater than 2%. In addition, we
746 identified the most abundant genera (N = 10) for each group based on overall mean relative abundances.
747 The number of *Batrachochytrium*-inhibiting taxa was calculated by blasting the rarefied dataset against the
748 AmphiBac-Database-2023.2 (Woodhams et al., 2015, updated by Bletz 2023); Antifungal database:
749 AmphibBac_InhibitoryStrict_2023.2; https://github.com/AmphiBac/AmphiBac-Database) and calculating
750 the average across all samples of a group.

751 All alpha diversity analyses were conducted with datasets rarefied to 90% of the read number of the sample
752 with the fewest reads in each comparison and visualized with boxplots. We compared richness, Shannon
753 index and evenness between different life stages of the same species, as well as between tadpoles from
754 different species and their environments. Differences in alpha diversity measures between groups were
755 determined using an ANOVA (for normally distributed, homoscedastic data) followed by a Tukey post-hoc
756 test or Kruskal-Wallis (for non-normally distributed data) followed by a Dunn test. P-values were adjusted
757 for multiple comparisons using Benjamini-Hochberg corrections.

758 We determined beta diversity across frog species, life stages, and aquatic environments using two
759 commonly used dissimilarity measurements as described for the captive-bred dataset. ASVs found in only
760 one frog were removed from the full, non-rarefied dataset. Variation was analyzed with a PERMANOVA
761 (function adonis within the package "vegan"), using Bray-Curtis and unweighted Unifrac dissimilarities as
762 responses and species, life stage, species- life stage interaction and parenting behavior as predictors.
763 Significant PERMANOVAs were followed by pairwise multilevel post-hoc comparisons between groups
764 with the function pairwise.adonis (package "pairwiseAdonis", Martinez Arbizu, 2020).

765 As previously suggested (Neu et al., 2021), we determined genus level agglomerated taxa between adults,
766 tadpoles and aquatic environments from unrarefied data converted to relative abundances across different
767 prevalence (prev) and abundance (abd) cutoffs. This approach avoids using arbitrary thresholds to define a
768 taxonomic 'core'. The following cutoffs were evaluated using the function "core_members" of the package
769 "microbiome" (v1.22.0, Lahti & Shetty, 2012, 2023): (1) prev 100% without abd cutoff, (2) prev >75%
770 with abd >1%, (3) prev >75% with abd >0.1% and (4) prev >75% without abd cutoff. We listed the most
771 abundant genera for each species (*Rv*, *Af* and *Ll*) and life stage (adult or tadpoles) with respect to their
772 presence or absence in the core of the aquatic environment of the respective species and used the package
773 "eulerr" (Larson, 2022) to visualize overlaps of genera with prevalence over 75% and relative abundance
774 over 0.1% with a Venn diagram.

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775 To test whether community distances between tadpoles and adults are smaller in poison frogs than in a
776 species without parental care, we performed a beta regression with an underlying logit function (R package
777 "betareg", Zeileis et al., 2021) to analyze unweighted Unifrac distances of a rarefied dataset. This approach
778 is suited to model predictions on our bounded distance dataset with high variance. ASVs were agglomerated
779 at the genus level prior to calculating distances between tadpoles and adults of different species, while
780 distances between related and unrelated pairs were calculated on a ASV level. To account for differences
781 in samples size between groups, we performed iterated analyses with random subsamples of equal sample
782 size (N = 8 for distances between adults and tadpoles between species and N = 10 for related vs. not-related
783 pairs of *Rv*) and report averaged p-values (following the approach of Hughey et al., 2017).

784 *Microbiota composition analyses of wild experimental tadpoles*

785 We determined and illustrated alpha and beta diversities of microbial communities on transported and non-
786 transported tadpoles and significant differences between the two groups as described for wild populations.
787 Variation was analyzed with a PERMANOVA (function adonis within the package "vegan"), using
788 unweighted Unifrac dissimilarity measures calculated from a rarefied dataset. Due to the limited sample
789 size and low biomass of some tadpoles, we chose to work with relative ASV abundances rather than
790 rarefaction to determine disparities and overlaps between the groups and their aquatic environment,
791 following previous examples (McMurdie & Holmes, 2013; Prest et al., 2018). Venn diagrams were created
792 without prevalence or abundance cutoffs and include all low abundance taxa. Differentially abundant
793 genera between transported and non-transported tadpoles were determined with the package
794 "ANCOMBC2" (version 2.2.2) (Lin, 2023).

795 We used 'Sourcetracker' (Knights et al., 2011) on unrarefied ASVs to determine proportions of the
796 communities of adult caregivers ("source") that can be detected on tadpoles that were collected from their
797 back and then reared in artificial cups for one month ("sink"). For this approach, we defined the
798 communities of each caregiver as source and transported tadpoles as sinks and reported minimum,
799 maximum and median source proportions detected among transported tadpoles.

800 ASVs shared between the transported tadpole and its transporting caregiver were identified using the
801 function "common_taxa" of the package "phylosmith" (Smith, 2023). We then evaluated the presence and
802 relative abundance of these shared taxa in transported and non-transported tadpoles, in their respective
803 aquatic cup environments, and in the water used to refill their cups. We converted relative abundances to
804 binary presence-absence information for illustration in a bubble chart and determined the ten taxa with the
805 highest relative abundance for each transporting caregiver individually from the unrarefied dataset. To
806 visualize the microbial interconnectedness of generationally shared genera within adult communities, we

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807 used the function "netConstruct" from the package NetCoMi (v1.1.0; Peschel et al., 2021) to construct
808 genus-level microbial networks of *Rv* adults (N = 44) using SPRING associations with a lambda index of
809 200 over 100 repetitions.

810 Permits

811 *Approval of the field monitoring by the scientific institution of the authors:*

812 The monitoring project in the Nouragues was reviewed by the Administrative Panel on Laboratory Animal
813 Care (APLAC) at Stanford University and approved under the APLAC-protocol 33691 (Protocol Director
814 LAO). The APLAC committee is Stanford's Institutional Animal Care and Use Committee (IACUC). It is
815 appointed by the University Vice Provost and Dean of Research.

816 *Approval from local field site:*

817 Non-invasive field experiments included in this publication were approved by the scientific committee of
818 the Nouragues Reserve. The field station in the Nouragues Reserve is managed by the CNRS (Centre
819 National de la Recherche Scientifique), is part of the USR 3456 LEEISA (Laboratoire Ecologie, Evolution
820 Interactions des Systèmes Amazoniens, founded in 2016) and located in the nature reserve 'Les Nouragues',
821 which is operated by the Office national des forêts (ONF) Guyane. Additional tissue collections were
822 approved by the Direction Générale des Territoires et de la Mer (DGTM approval number # R03-2022-08-
823 10-00001).

824 Data availability

825 Data files and data analysis scripts used to generate the results are available on DataDryad (pending
826 acceptance).

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832 statistical consultation and Philippe Gaucher for endless knowledge, discussions and guidance in the world
833 of amphibians. This research was conducted at Stanford University, which is located on the ancestral and

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834 unceded land of the Muwekma Ohlone tribe. Our field work was conducted in the Nature Reserve 'Les
835 Nouragues' in French Guiana, that was founded on land ancestrally inhabited by the Amerindien Nouragues
836 ('Norak') tribe.

837 ## Declaration of Interests

838 The authors declare no competing interests.

839 ## Author Contributions

840 Conceptualization: MTF, SC
841 Data Curation: MTF, KSX, EKC, MD, GR, AR
842 Formal Analysis: MTF, KSX, EKC, MD
843 Investigation: MTF
844 Visualization: MTF, LAO
845 Methodology: MTF
846 Writing: MTF
847 Review and Editing: KSX, EKC, DAR, LAO
848 Project Administration: MTF, LAO
849 Supervision: KSX, EKC, SC, DAR, LAO
850 Resources: DAR, LAO
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