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The effect of captivity on the cutaneous bacterial community of the critically endangered Panamanian golden frog (*Atelopus zeteki*)

Matthew H. Becker [a,*], Corinne L. Richards-Zawacki [b], Brian Gratwicke [c], Lisa K. Belden [a]

[a] Department of Biological Sciences, Virginia Tech, Blacksburg, VA, USA
[b] Department of Ecology and Evolutionary Biology, Tulane University, New Orleans, LA, USA
[c] Smithsonian Conservation Biology Institute, National Zoological Park, Washington, DC, USA

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ABSTRACT

For many threatened vertebrates, captivity may be the only option for species survival. Maintaining species in captivity prior to reintroduction presents many challenges, including the need to preserve genetic diversity and mitigation of disease risks. Recent studies suggest that captivity can alter the suite of symbiotic microbes that play important roles in host health. The Panamanian golden frog (*Atelopus zeteki*) has not been seen in its native habitat in Panamá since 2009. Along with habitat loss and illegal collecting, the lethal disease chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), is responsible for the severe decline of this species. Prior to the spread of Bd into golden frog habitat, conservation organizations collected golden frogs and placed them in captive survival assurance colonies. The skin of amphibians is host to a diverse resident bacterial community, which acts as a defense mechanism in some amphibians to inhibit pathogens. We characterized the cutaneous bacterial community from wild and F1 captive golden frogs originating from the same population with Illumina sequencing to assess how long-term captivity has affected this community. We found that species richness, phylogenetic diversity, and community structure of the skin microbiota was significantly different between wild and captive golden frogs. However, after approximately eight years of living in captivity, the offspring of the original captive golden frogs still shared 70% of their microbial community with wild frogs. These results demonstrate that host-associated microbial communities can be significantly altered by captive management, but most of the community composition can be preserved.

1. Introduction

Over the past few decades, we have seen a disturbing rate of species declines and extinctions due to a variety of factors including habitat loss, disease, and climate change (Heard et al., 2013; Stuart et al., 2004). It has even been suggested that we are currently witnessing a sixth mass extinction (Wake and Vredenburg, 2008). For many threatened species, captivity is the only tool available to conservation managers to prevent extinction when survival in the organism's native habitat is not possible, as is the case with the Panamanian golden frog, *Atelopus zeteki* (Gagliardo et al., 2008). However, managing species under captive conditions cannot only permanently deteriorate the host's genome (Woodworth et al., 2002), but also alter symbiotic microbial communities associated with these organisms. Symbiotic microbial communities of many wild species including monkeys, bears, seals, grouse, parrots, sponges, and salamanders have been affected while these animals have been kept in captivity (Loudon et al., 2013; Nakamura et al., 2011; Nelson et al., 2013; Schwab et al., 2011; Webster et al., 2011; Wienemann et al., 2011; Xenoulis et al., 2010).

With advancements in molecular and microbial techniques, we are now discovering how vital symbiotic microorganisms are to the health and normal function of the host they inhabit. For example, microbial communities associated with the human gut facilitate metabolic and absorptive processes and stimulate immunity (Bäckhed et al., 2005; Fujimura et al., 2010). In addition, symbiotic microbes in some species, including *Atelopus*, may produce toxins (e.g. tetrodotoxin) that protect the host from predators (Chau et al., 2011). One possible contributing factor to the low historical success rate of reintroductions with endangered species (11–53%; Beck et al., 1994; Fischer and Lindenmayer, 2000; Wolf et al., 1996) is that captive rearing alters the host's microbial community, decreasing subsequent survival of the animal in the wild (Redford

* Corresponding author. Address: Department of Biological Sciences, 2119 Derring Hall, Virginia Tech, Blacksburg, VA 24061, USA. Tel.: +1 540 231 5854.
E-mail addresses: beckermh@vt.edu (M.H. Becker), cori@tulane.edu (C.L. Richards-Zawacki), gratwickeb@si.edu (B. Gratwicke), belden@vt.edu (L.K. Belden).

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et al., 2012). For example, many attempts to reintroduce the grouse *Tetrao urogallus* have failed (Seiler et al., 2000) likely due to impaired digestion as a result of anatomical changes in the gut (Liukkonen-Anttila et al., 2000) and shifts in the gut microbial community as a result of captive management (Wienemann et al., 2011). While much of this work has focused on birds and mammals, it seems likely that host-associated microbial communities also contribute to the success of amphibians in their native habitats and could be an important component of successful amphibian reintroduction programs.

Along with most species in the genus *Atelopus*, the Panamanian golden frog is critically endangered (Lips et al., 2010) and it has not been seen in the wild since 2009, despite intensive search efforts (E. Griffith, personal communication; La Marca et al., 2005; C.L.R.-Z., unpublished data). The frog's historical range was in a small area of central-western Panamá. Chytridiomycosis, a disease caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), has been spreading through Panamanian amphibian assemblages in a south-easterly direction for almost two decades and has caused severe population declines and extinctions (Cheng et al., 2011; Lips et al., 2006; Woodhams et al., 2008). In one documented case in Panamá, approximately 50% of the amphibian species and 80% of the individuals disappeared in a few months following the initial detection of Bd (Lips et al., 2006). In response to the declines of related *Atelopus* species (e.g., *Atelopus varius*) and prior to the spread of Bd into the historical range of *A. zeteki*, Project Golden Frog (http://www.projectgoldenfrog.org), in collaboration with multiple zoos in the United States, collected and placed approximately 100 *A. zeteki* in captive survival assurance colonies (Gagliardo et al., 2008). Currently, over 2000 *A. zeteki* are being maintained in North American and Panamanian zoos and aquaria (K. Murphy, personal communication; Poole, 2008). The ultimate goal of this *ex situ* conservation program is to reintroduce *A. zeteki* back to their native habitat in Panamá. Unfortunately, *A. zeteki* is highly susceptible to chytridiomycosis. Bd still remains in the environment and on less susceptible amphibian species, so it is unlikely that any Bd-free environments exist (Becker et al., 2012). Reintroduction of *A. zeteki* will therefore require Bd mitigation strategies, such as the use of beneficial bacteria (probiotics; Becker et al., 2009; Harris et al., 2009; Becker and Harris, 2010; Bletz et al., 2013).

Because cutaneous bacteria have important health-related functions for amphibian hosts and because the use of these bacteria in probiotic-based mitigation strategies is possible, it is important to determine how captivity affects these complex bacterial communities. Many environmental factors, such as humidity, temperature, and pH, affect skin or surface-associated microbial communities in animals (McBride et al., 1977; Meron et al., 2011; Webster et al., 2008). In addition, a lack of natural environmental reservoirs of bacteria can also alter the composition of host-associated microbial communities (Loudon et al., 2013). Therefore, frogs reared in a captive environment likely have different cutaneous bacterial communities than individuals in wild populations. The aims of this study were to characterize the historical symbiotic bacterial communities associated with the skin of wild *A. zeteki* and to examine the effects of long-term captive management on the structure of these communities.

2. Materials and methods

2.1. Study species and sites

We characterized and compared the cutaneous microbial community structure from both a wild and a captive population of *A. zeteki*. Samples were collected by swabbing back and forth 3–5 times on the surfaces of each the venter, dorsum, thighs, and feet of each frog with a sterile swab, as to sample the entire surface. We changed gloves for the handling of each frog. Wild adult *A. zeteki* ($N = 27$) were sampled from a population located near Río Mata Ahogado in Panamá in 2005 and 2006, and released at the site of capture after swabbing (Richards-Zawacki, 2010). These swabs were stored at room temperature in a salt-saturated DMSO solution prior to DNA extraction. Captive adult *A. zeteki* ($N = 10$) were sampled from a population at the Smithsonian National Zoological Park in Washington, D.C. in 2011. These individuals were born in captivity in 2005 from parents that were collected in 2003 from the same population from which wild samples were collected. After laying eggs, parents were removed from enclosures and had no further contact with offspring. We stored swabs from captive frogs at −80 °C prior to DNA extraction. DNA preservation technique (other than filter card techniques) and length of time in storage does not significantly affect the assessment of microbial community structure (Dolfing et al., 2004; Gray et al., 2013; Lauber et al., 2010).

2.2. Sample preparation and sequencing

We extracted DNA from each swab with PrepMan Ultra (Applied Biosystems, Carlsbad, California) following methods outlined by Hyatt et al. (2007). This DNA extraction method is optimized for the extraction of DNA from Bd, but it is also effective at extracting DNA from prokaryotic cells. The V4 region of the 16s rRNA gene was amplified with PCR and the primers 515F and 806R (Caporaso et al., 2010b). The reverse primers contained a 12 base error-correcting Golay code (Fierer et al., 2008), which we used to uniquely tag PCR products of each sample. We prepared PCR reactions as described by Costello et al. (2009). Briefly, triplicate reactions of each sample contained 1 μl template DNA, 12 μl DNA-free PCR water (MO-BIO, Carlsbad, California), 10 μl 2.5× HotMasterMix (5 PRIME, Gaithersburg, Maryland), 1 μl of 20 mg/ml bovine serum albumin (Fisher Scientific, Pittsburgh, Pennsylvania), and 0.5 μl of each primer at 10 μM concentration. We ran controls without template for each sample. DNA extracted from a sterile swab was also included as a negative control. We diluted extracted DNA samples that contained PCR inhibitors 1:10 in PCR water. The amplification conditions were as follows: an initial cycle for 3 min at 94 °C followed by 35 cycles of 34 s at 94 °C, 60 s at 50 °C, and 90 s at 72 °C, with a final cycle for 10 min at 72 °C. Amplification conditions for five samples with low DNA concentrations were altered to include 38 cycles. Triplicate reactions of each sample were pooled, visualized on a 1% agarose gel, and quantified with a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, California). We purified PCR products with the Qiagen QIAquik PCR Purification Kit (Qiagen, Valencia, California) using the manufacturer's protocol. An equimolar mixture of all the samples was then sequenced on an Illumina MiSeq instrument (San Diego, California) with a 250 bp paired-end strategy at the Dana-Farber Cancer Institute, following methods similar to those in Caporaso et al. (2012). To compensate for the low base diversity of the amplicon pool, the sample was run with a 10% PhiX control. Version 1.18.42 of the MiSeq Real-Time Analysis software (Illumina) was used to perform base calling and quality scoring.

2.3. Sequence data processing

Sequence data were assembled with Fastq-join (https://www.code.google.com/p/ea-utils/wiki/FastqJoin) with default parameters and processed with the Quantitative Insights Into Microbial Ecology pipeline (QIIME v. 1.7.0; Caporaso et al., 2010a). We clustered quality-filtered sequences into distinct bacterial OTUs (operational taxonomic units) at a sequence similarity threshold of 97% and assigned taxonomy with RDP classifier and the

Greengenes database. All samples were rarefied to 19,500 sequences to standardize sampling effort. Details of the bioinformatics methods are in Appendix A.

2.4. Statistical analysis

Unless noted, all dependent variables were normally distributed and variances were equal among specific comparisons. We computed measures of alpha diversity (within-sample diversity), including OTU richness, phylogenetic diversity, and Shannon diversity index, with QIIME. We used Student's *t*-tests to test for significant differences in alpha diversity measures between the wild and captive populations. To compare the microbial community structure between samples, a Bray–Curtis distance matrix (Bray and Curtis, 1957) was built on square-root transformed data with the software package Primer 6 (version 6.1.15). We completed all further community composition comparisons with Primer 6 and Permanova + (version 1.0.5). From the distance matrices, differences in community composition between the wild and captive populations were statistically analyzed with Analysis of Similarity (ANOSIM) and visualized with principal coordinates analysis (PCO). Relative abundances of phyla, genera, and individual OTUs were not normally distributed; therefore, we statistically analyzed differences between populations with Wilcoxon rank-sum tests. We corrected all multiple comparisons with the false discovery rate procedure (FDR; Benjamini and Hochberg, 1995). The core microbiota was defined as OTUs that were present on 90% or more of individuals in each population. A phylogenetic tree was built to visualize the distribution of OTUs among dominant phyla that were shared and unique to each population. The tree was constructed with MUSCLE aligned sequences (Edgar, 2004) using FastTree (Price et al., 2009) and visualized with the Interactive Tree of Life (Letunic and Bork, 2007). The phylogenetic tree is not meant to portray specific evolutionary relationships among individual OTUs.

3. Results

3.1. Alpha diversity (within-sample diversity)

There was a large amount of variation in the diversity of skin communities among individual *A. zeteki* in both wild and captive populations (Fig. 1). For example, the number of OTUs (OTU richness) on wild frogs ranged from 136 to 1451 OTUs per frog (Fig. 1a). Despite the large individual variation, OTU richness and phylogenetic diversity were significantly higher in captive *A. zeteki* than in wild frogs (Fig. 1a, *t*-test, $P < 0.01$ and Fig. 1b, *t*-test, $P < 0.01$, respectively). However, wild *A. zeteki* had a significantly higher Shannon diversity index (a measure of evenness) than captive individuals (Fig. 1c, *t*-test, $P = 0.02$).

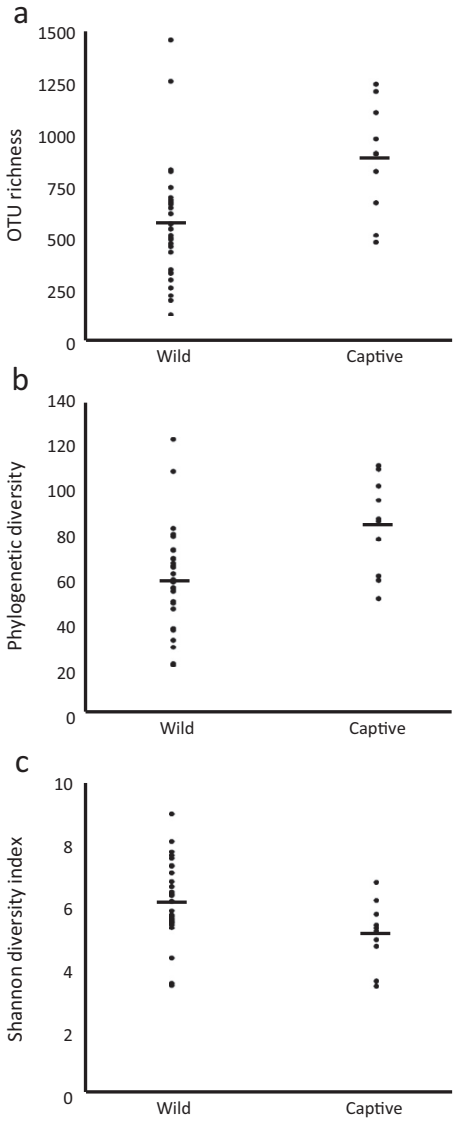

Fig. 1. Alpha diversity (within-sample diversity) of skin-associated microbial communities present on captive and wild *Atelopus zeteki*. (a) The number of unique operational taxonomic units (OTUs) at a 97% sequence similarity in each community. (b) Phylogenetic diversity is a measure of the total branch length of a phylogenetic tree covered by a community. (c) Shannon diversity index is a measure of richness and evenness of OTUs in a community. Each point represents a community on an individual frog. Horizontal lines represent sample means.

3.2. Community composition differences and shared microbiota

Although there was a considerable amount of variation in community composition among the microbial communities of frogs within each population, the variation between captive and wild populations was strikingly different (ANOSIM, Global $R = 0.443$, $P = 0.001$) and formed two distinct clusters on a PCO plot (Fig. 2). However, the offspring of individuals that were placed in captive assurance colonies in 2003 still shared 2137 OTUs with wild *A. zeteki* (Fig. 3a). When considering only shared OTUs, community structure between the captive and wild populations was still significantly different (ANOSIM, Global $R = 0.416$, $P = 0.001$) because the relative abundances of these shared OTUs differed between populations.

Shared OTUs were dominant members in the communities on both wild and captive *A. zeteki* when compared to OTUs only found in only one population (Fig. 3b). For example, the mean relative abundances of the most abundant shared OTU in wild and captive populations (10% and 21%, respectively) was two orders of magnitude higher than the most abundant OTU present only in the wild or captive population (0.37% and 0.30%, respectively). The core microbiota (OTUs present on $\geqslant$90% of individuals) of the wild population consisted of 11 OTUs (Table A1), and these were also shared with the captive population. Ten out of the 11 were present on 100% of the captive frogs, with the remaining OTU present on 80% of the captive frogs.

3.3. Microbiota unique to each population

Although the wild and captive populations shared many OTUs, there were 2856 OTUs unique to the wild population and 915

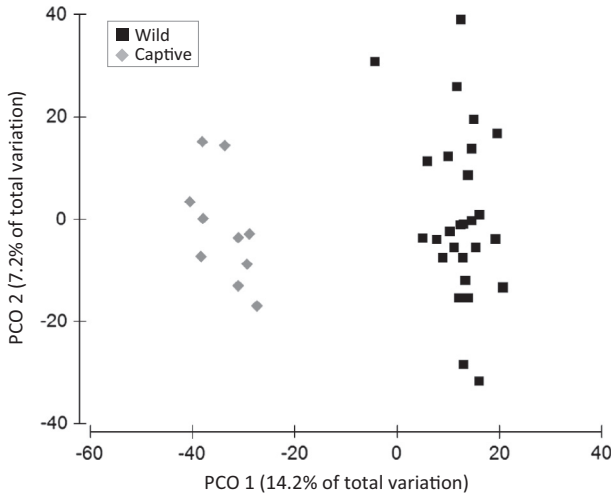

Fig. 2. Principal coordinate plot of Bray–Curtis distances between microbial communities present on wild and captive *Atelopus zeteki*. Each point represents a microbial community of an individual frog.

unique to the captive population (Fig. 3a). A majority of these OTUs were at low prevalence (proportion of individuals that have a particular OTU) in both populations. Of the OTUs unique to wild frogs, 98% (2663/2856) had a prevalence ⩽30% (Table A2), while of the OTUs unique to captive frogs, 77% (707/915) had a prevalence ⩽30%. OTUs unique to each population were distributed throughout all the dominant bacterial phyla present on wild *A. zeteki* (Fig. 3d).

3.4. Dominant bacterial phyla and families

The most dominant phyla (mean relative abundance >0.05% in either population) in the populations were Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Planctomycetes, Proteobacteria, and Verrucomicrobia (Fig. 3c). These eight phyla were present on >90% of the frogs in both populations. Only three phyla had mean relative abundances that were significantly different between the wild and captive populations (Wilcoxon rank-sum test, FDR-corrected $P < 0.05$). The phylum Bacteroidetes had a mean relative abundance of 27% and 43% on wild and captive *A. zeteki*, respectively. An individual OTU in the genus *Pedobacter* (Family: Sphingobacteriaceae) accounted for 97.6% of this difference. This OTU was present on all captive and wild individuals in the study and had the highest mean relative abundance across all samples. The phyla Planctomycetes and Verrucomicrobia were significantly more abundant on wild frogs. There were 35 dominant bacterial families (mean relative abundance >0.05%) on *A. zeteki*, which are shown in Fig. A1 with their respective relative abundances.

3.5. Dominant operational taxonomic units (OTUs, ~bacterial "species")

Many dominant members of the microbial community (OTUs with a mean relative abundance >0.5%) were also members of the core microbiota (present on ⩾90% of individuals) in each population (Fig. 4). Dominant OTUs were all classified in the phyla Bacteroidetes, Actinobacteria, or Proteobacteria. There were 15 dominant OTUs present on wild *A. zeteki* and seven of these OTUs were core members of the microbiota. The relative abundances of nine of these 15 dominant wild frog OTUs were significantly different in the captive population (Wilcoxon rank-sum test, FDR-corrected $P < 0.05$). Six of them were lower in abundance on captive individuals and three were higher in abundance on captive individuals. Those that had lower abundances on captive frogs were drastically lower. For instance, an OTU classified as belonging to the family Actinomycetales had a mean relative abundance of 3.9% on wild frogs and only 0.03% on captive individuals, despite having a prevalence of 100% in both populations. The most dominant OTU in both wild and captive frogs (*Pedobacter*, discussed in Section 3.4) doubled in relative abundance on captive frogs, thus skewing the OTU relative abundance distribution in these frogs. This likely caused the captive population to have a significantly lower Shannon diversity index (= less even community) than the wild population (Fig. 1c). There were also four OTUs that had low abundance on wild frogs, but were dominant on captive frogs (Wilcoxon rank-sum test, FDR-corrected $P < 0.05$). These OTUs also increased in prevalence in the captive population.

4. Discussion

From 2001 to 2005, *A. zeteki* from Panamá were collected from their native habitats and placed in captive assurance colonies prior to the invasion of Bd (Gagliardo et al., 2008). Our results indicate that the skin microbiota of F1 captive *A. zeteki* was significantly different than wild frogs, in terms of species richness, evenness, phylogenetic diversity, and community composition. This same pattern has been seen in other animals managed under long-term captive conditions (Isaacs et al., 2009; Nakamura et al., 2011; Nelson et al., 2013; Schwab et al., 2011; Wienemann et al., 2011; Xenoulis et al., 2010). For example, OTU richness was much higher in the surface-associated microbiota of captive sponges (Mohamed et al., 2008) and in the gut microbiota of captive seals (Nelson et al., 2013) and parrots (Xenoulis et al., 2010) than in their wild counterparts, which was also observed for golden frogs in the present study. However, other studies have shown the converse (higher diversity in wild animals; Isaacs et al., 2009; Nakamura et al., 2011).

Some changes in *A. zeteki* microbiota during captive management are likely due to environmental factors such as humidity, temperature, and pH (McBride et al., 1977; Meron et al., 2011; Webster et al., 2008). Captive *A. zeteki* were kept under conditions that resemble their natural habitat. However, it is impossible to simulate in captivity the variety of microhabitat conditions that these frogs experienced in the wild. In addition, the potential for transmission of bacteria from other sources is increased in captivity (Nelson et al., 2013) and may explain why captive frogs had higher richness and phylogenetic diversity than wild frogs. This could happen by co-habitation of several *A. zeteki* in the same enclosure, by interaction with the microbiota of zookeepers, and by exposure to the microbiota of other frog species (through environmental transmission) and microbes living on environmental substrates in enclosures (plants, rocks, soil, and water).

There is also a concern that long-term managed species, with multiple generations born in captivity, are likely to experience permanent microbiota changes due to host factors if genetic variation cannot be preserved. For instance, mutations in genes associated with the immune system can result in changes to the structure of gut-associated microbial communities of mice and humans (Spor et al., 2011). Minimizing time managed under captive conditions may reduce changes to the microbial community. Sponges placed in captive conditions for short periods of time (<6 months) had very similar surface microbial communities to wild-caught sponges (Gerçe et al., 2009; Webster et al., 2011). However, after 12 months in captivity, sponges had a very different symbiont community structure than wild-caught sponges with many wild-associated microbes lost and new or rare members becoming dominant (Webster et al., 2011).

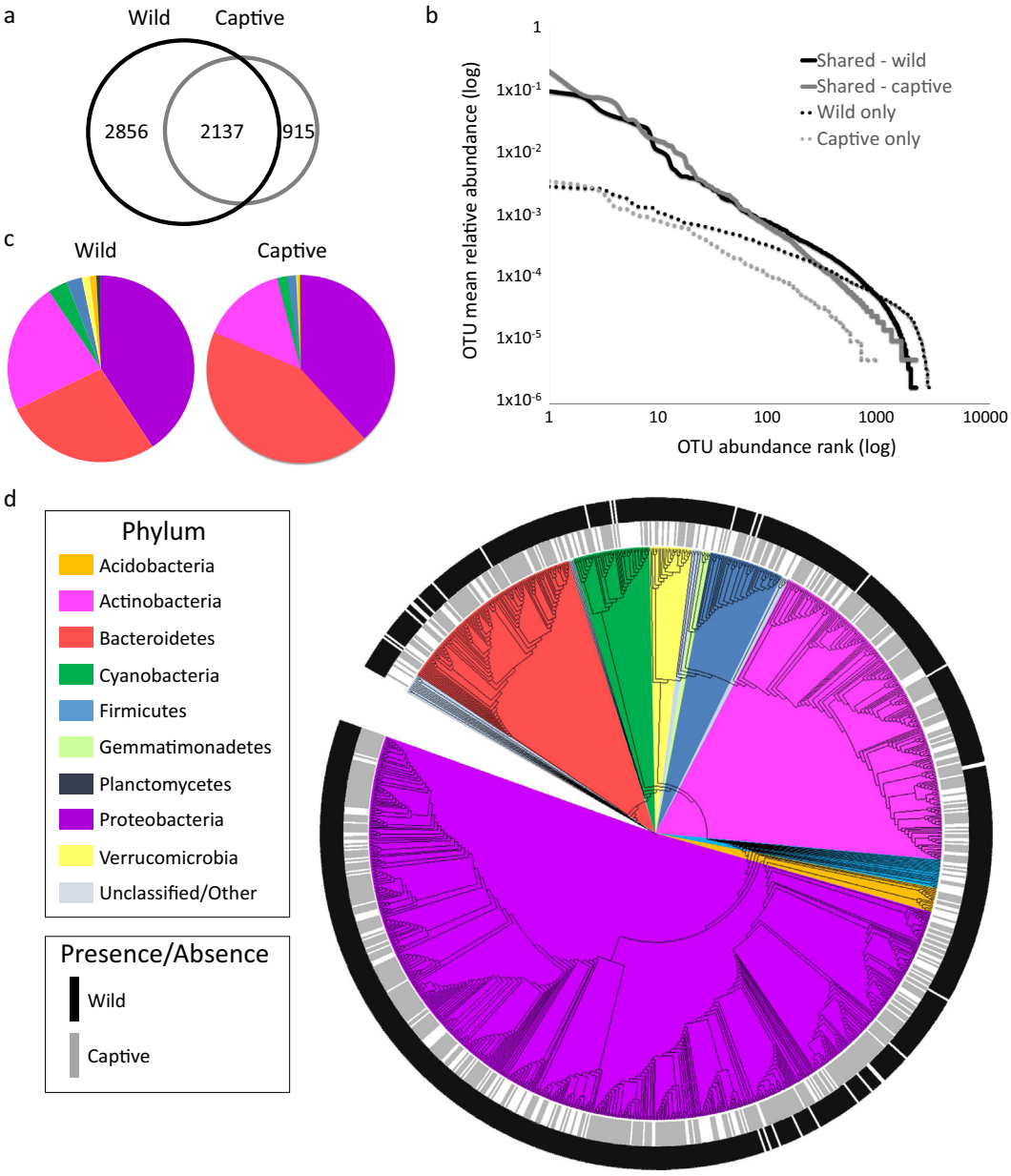

Fig. 3. Comparison of shared and unshared operational taxonomic unit (OTUs) as well as the taxonomic diversity of the microbial community present on wild and captive *Atelopus zeteki*. (a) Venn diagram displaying the distribution of shared and unshared OTUs present on wild and captive populations of *A. zeteki*. (b) Rank abundance curves of the OTUs in each section of the Venn diagram. (c) Pie charts displaying the mean relative abundance of phyla from each frog population that had a relative abundance greater than 0.05% on either wild or captive *A. zeteki*. (d) Phylogenetic tree constructed from OTUs that had a mean relative abundance greater than 0.01% on wild and captive *A. zeteki*. The branch color corresponds to bacterial phyla present. The inner and outer rings refer to the presence of individual OTUs in captive and wild *A. zeteki*, respectively.

Although there were significant differences between the microbial communities of wild and captive golden frog populations, 70% of the OTUs on captive frogs were shared with wild frogs. In addition, all but one core bacterial species of wild *A. zeteki* were also core members of the microbial community of captive frogs. This suggests that even in captivity, the primary symbionts may be maintained over generations. However, the relative abundances of most shared OTUs were drastically different between populations where, with a few exceptions, OTUs abundant on wild frogs were rare on captive frogs and vice versa. So even though the species in the microbial communities were largely shared, the community structure differed significantly between the two populations with a more even community in the wild population and a community dominated by fewer taxa in the captive population.

The fact that a majority of the microbes were retained in captivity suggests that either these microbes are transmitted by vertical or pseudo-vertical transmission or that they are abundant in a broad range of environments. Vertical transmission occurs when microorganisms are transferred from parent to offspring (Bright and Bulgheresi, 2010). This seems unlikely since *A. zeteki* parents had no contact with offspring after laying eggs. However, a study of the glass frog *Hyalinobatrachium colymbiphyllum* suggests that skin bacteria can be vertically transmitted from amphibian parents to embryos in some species (Walke et al., 2011). We hypothesize pseudo-vertical transmission, in which microorganisms are transmitted from parent to offspring through an intermediate environmental source, is the more likely mode of transmission for captive *A. zeteki*. For instance, bacteria from the skin of parents could be

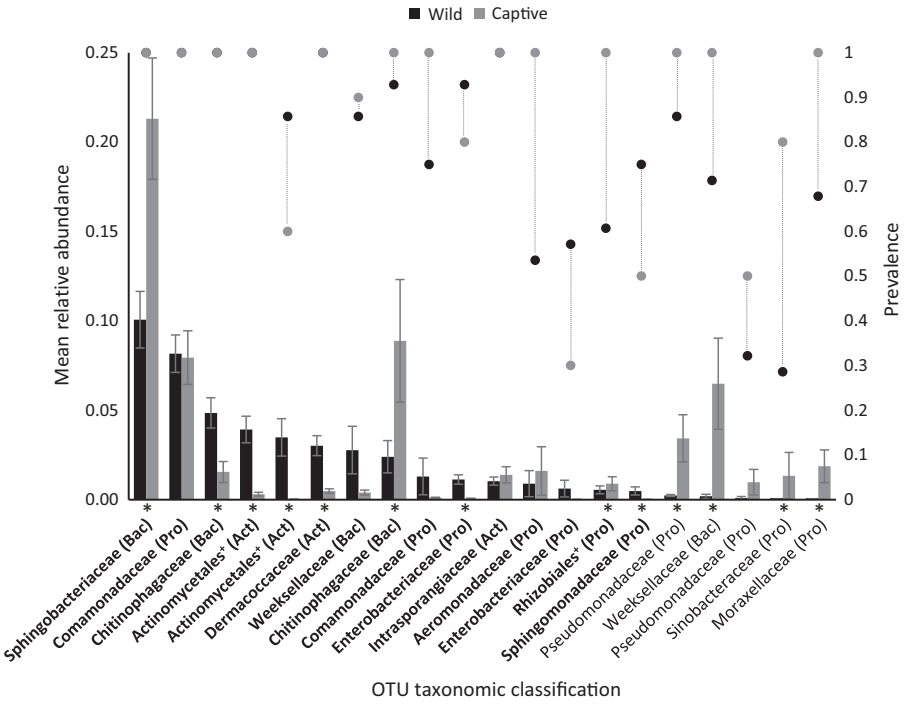

Fig. 4. Mean relative abundance (bars) and prevalence (closed circles) of dominant bacteria present on wild and captive *Atelopus zeteki*. Only operational taxonomic units (OTUs) with a mean relative abundance greater than 0.5% in either population are shown (bolded OTU labels represent dominant OTUs of wild frogs). The family (or order when denoted by an addition sign) and phylum (in parentheses) for each OTU are shown on the *x*-axis. Error bars represent standard error. Wilcoxon rank-sum test FDR-corrected $P < 0.05$ denoted by star. Bac = Bacteroidetes; Pro = Proteobacteria; Act = Actinobacteria.

transmitted to the water within the enclosure. These bacteria could remain in the enclosure after the parents are removed and colonize the skin of offspring.

At the phylum level, there were many similarities in the relative abundances of the dominant phyla present on wild and captive golden frogs, with the exception of three phyla (Bacteroidetes, Planctomycetes, and Verrucomicrobia). Bacteroidetes was significantly more abundant on captive frogs due to the increase in relative abundance of a single *Pedobacter* species (Family: Sphingobacteriaceae). The increase in relative abundance of this single OTU also likely drove the decrease in evenness in the captive population, despite the greater richness and phylogenetic diversity in the captive population. This *Pedobacter* sp. was the most abundant OTU in both populations and its mean relative abundance more than doubled on captive frogs, resulting in a more skewed distribution. This increase may be due to the ability of this organism to more successfully grow and compete than other bacteria in the microhabitat created by captive conditions. Species of *Pedobacter* are commonly found on the skin of amphibians (Harris et al., 2006; Lam et al., 2010; Lauer et al., 2008). The phyla Planctomycetes and Verrucomicrobia were more abundant on wild frogs. Phylogenetic analysis suggests these two phyla are closely related (Hou et al., 2008) and are commonly found in a variety of aquatic habitats and in association with animals. Verrucomicrobia are also commonly found in soils (Wagner and Horn, 2006). Therefore, the decrease in abundance of these phyla on captive individuals may be explained by the lack of transmission from native environmental sources. It is difficult to determine the proportion of amphibian resident bacteria that are derived from the environment, but recent studies have reported that 16–90% of the cutaneous bacteria are shared with the amphibian's surrounding environment and may be species-dependent (Kueneman et al., 2013; Loudon et al., 2013; Walke et al., 2014).

Although there were many similarities between wild and captive frogs at the phylum level, the relative abundances of many bacterial families were strikingly different between populations (Fig. A1). Interestingly, the bacterial families that had higher relative abundances on captive frogs (Cellulomonadaceae, Flavobacteriaceae, Moraxellaceae, Neisseriaceae, Nocardiaceae, Pseudomonadaceae, Sanguibacteraceae, and Sphingobacteriaceae) have also been commonly found in abundance on North American amphibians (McKenzie et al., 2012; Walke et al., 2014). These results suggest that either environmental conditions of captive *A. zeteki* favored the growth and reproduction of these families, or that OTUs in these families were indirectly transmitted from other amphibians or environmental sources while in captivity in North America.

Overall, the results of our study demonstrate that captive management can significantly alter the structure of the microbial community on *A. zeteki*. Important next steps in this line of research include investigating how the reintroduction of golden frogs to their native habitat will likely affect their skin-associated microbial community. If golden frog microbiota are obtained through environmental sources and mediated through environmental factors, then it is possible that the pre-captivity microbial community composition and structure will be recovered once they are returned to Panamá. However, if golden frogs rely on vertical or pseudo-vertical transmission then bacterial species lost in captivity may never recover. As noted earlier, host-associated microbial communities provide many vital functions to the host and changes to this microbiota may have severe consequences for reintroduction efforts of *A. zeteki*, such as increased susceptibility to endemic or recently emerged pathogens (Schommer and Gallo, 2013). Therefore, it may be important to conserve the microbial diversity of captive species, as well as the genetic diversity, if the goal of captive management is reintroduction.

One approach to prevent alterations to host-associated microbiota in species that have environmentally derived microbiota is to provide native environmental sources in their captive enclosures, although care must be taken not to introduce pathogens with these items. For example, a recent study demonstrated that captive

management of the red-backed salamander (*Plethodon cinereus*) with native soils present in their enclosures reduced changes to their microbial community when compared to more sterile rearing conditions (Loudon et al., 2013). If microbial communities are largely derived through vertical transmission, then cohabitation of parents and offspring would largely reduce changes of the microbiota. In captive populations, it may also be critical to limit the use of antibiotics, which can have long-lasting and possibly permanent effects to the microbiota (Lozupone et al., 2012). Although our study is limited to the bacterial community associated with amphibians, it is likely that captive management affects other symbiotic microbiota, such as fungi and viruses. For instance, the use of antifungal treatments, which are important for treating and preventing Bd in captive amphibians (Georoff et al., 2013), could also affect symbiotic fungi and/or alter microbial interactions in these complex communities.

When the microbial community of a host is viewed as an extension of the host's genetic makeup and ability to adapt (Rosenberg et al., 2007), it becomes clearer that preserving the diversity of host-associated microbiota may be important for the success of future reintroduction efforts and the long-term persistence of species, including the Panamanian golden frog. Studies investigating how changes in host-associated microbial communities due to captive management affect host function and disease resistance may be critical when developing a successful reintroduction program for endangered species.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocon.2014.05.029.

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