Introduction

We are only now beginning to understand that individual animals are not solely discrete entities, but they in fact host a plethora of symbiotic microbes that can have significant effects [1–3]. The realization of the importance of the roles that microbial symbionts play in eukaryotic life is a source for excitement and is leading to a more complete understanding of evolution [4–7]. Host-microbiota interactions are essential for many facets of physiology, ranging from metabolic activity to immune homeostasis [8,9]. In the vertebrate gut, bacteria are known to play important physiological roles that influence metabolic processes, such as the digestion of complex carbohydrates [10] and the regulation of fat storage [11]. It has recently been shown that gut microbiota composition can also influence behavior and gene expression in key brain regions in mice, with motor control and anxiety-like behavior differing between mice with and without their normal gut microbiota [12]. The importance of this microbiome-gut-brain-axis is becoming increasingly evident, and differences in bacterial community composition have been shown to affect emotional, learning and memory behavior, and problem solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15]. Microbes might even play a role in speciation in eukaryotes [13–15]. The attention of speciation solving abilities in mammals [13–15].
identical diets in the laboratory since then. We aimed to establish a base line for host-microbiota studies of cichlid fish and to ask whether the gut microbiota differed between ecomorphs [28]. If benthic and limnetic species pairs that arose independently, and live in different lakes [29] show parallel differences across lakes, this would speak for an adaptive value of a particular microbiota relating to similarities in their feeding ecology.

Material and Methods

Sample collection and preparation

For the present study, we used a total of 12 fish, three of each of the four species: *A. astorquii* (benthic species from Lake Apoyo), *A. zaliosus* (limnetic species from Lake Apoyo), *A. amarillo* (benthic species from Lake Xiloá) and *A. sagittae* (limnetic species from Lake Xiloá). Fish were collected in the wild as fry in 2005 with permission from MARENA and raised under identical conditions at the University of Konstanz animal facility.

The 12 fish chosen for the present study were selected from the above-mentioned wild-caught lab-reared stock based on being as similar as possible in size (standard length $178 \pm 15$ mm) and being the same sex (only female specimens were used) to remove potentially confounding factors from the analysis. Each fish was sacrificed with an hypothermic treatment and immediately dissected under a hood. A sample of about 200 mg was excised from the gut frontal to the junction between the esophagus and the stomach.

DNA extraction, amplification and sequencing

DNA extractions were performed with the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, USA) following the manufacturer's protocol. The integrity of each DNA sample was assessed using agarose gel electrophoresis and quantified using a Qubit v2.0 fluorometer (Life Technologies, Darmstadt, Germany). Illumina libraries were prepared following the method described by Caporaso et al. [30] using the NEXTflex 16S V4 Amplicon-Seq Kit (Bioo Scientific, Austin, USA). Briefly, from 50 ng of DNA template for each sample, the bacterial V4 region of the 16S ribosomal gene was amplified using the universal primers 515F and 806R tailed with Illumina barcoded adapters [26] following the PCR conditions recommended by the manufacturer. PCR products were purified using the Agencourt XP Ampure Beads (Beckam Coulter, Inc.) and, subsequently, aspecific DNA fragments were removed using the MinElute Gel Extraction Kit (Qiagen). The quality of the final products was assessed using a Bioanalyzer 2100 (Agilent Technologies, Waldbronn, Germany) and, after their quantification with a Qubit, the samples were pooled in equal proportions and sequenced paired-end in an Illumina MiSeq with 312 cycles (151 cycles for each paired read and 10 cycles for the barcode sequence). To prevent focusing, template building and phasing problems due to the sequencing of “low diversity” libraries such as 16S amplicons, 50% PhiX genome was spiked in the pooled library. All sequences have been deposited in the NCBI's Sequence Read Archive (SRA accession number to be provided upon acceptance).
Sequence processing

SeqPrep (https://github.com/jstjohn/SeqPrep) was used to remove any reads that were contaminated with Illumina adapters and to merge overlapping paired-end reads into single longer reads covering the full 16S V4 region (254 bp). To avoid the generation of incorrect sequences, the minimum overlapping length was set to 15 bp. Next, the resulting sets of paired- (merged) reads were filtered for quality using CLC Genomics Workbench v6.5 (CLC bio, Aarhus, Denmark). Low quality reads (CLC “limit” set to 0.01) were discarded. To improve taxonomic assignment, only reads representing the full 16S V4 region (252 to 254 bp) were retained for downstream analyses.

Characterization of the microbial communities of the studied species

For a general characterization of the main microbial taxa found in the four cichlid fish species studied here, we used the Visualization and Analysis of Microbial Population Structures (VAMPS, http://vamps.mbl.edu/) webservice. Next-generation processed reads were clustered into Operational Taxonomic Units (OTUs) using a 97% identity threshold with USEARCH [31] and then information on the most representative taxa were extracted. We also performed a principal coordinate analysis based on the matrix of pairwise Euclidean distances between samples obtained in MG-RAST [32] using the lowest common ancestor method (LCA; [33]) for taxonomic annotation.

Analysis of the difference in microbial composition between benthic and limnetic fish

To test if benthic and limnetic Midas cichlids are characterized by different microbial communities we used a two-step approach. First, we tested for overall difference in microbial communities between ecomorphs, then we identified the relevant bacterial taxa by performing taxonomic assignment on sequences whose number of reads were different between benthic and limnetic species. For the analysis of the overall difference in microbial communities we performed the permutation test with UniFrac weighted distances [34] implemented in QIIME [30] both comparing benthic and limnetic fish pooled between lakes and performing separate comparisons for *A. aslongui* vs *A. zahosui* and *A. amarillo* vs *A. sagittae*.

To identify bacterial taxa present differentially in benthic and limnetic fish, we extracted the raw counts of the number of sequences that were assigned to each of the OTUs obtained with the USEARCH clustering algorithm at a 97% similarity threshold. Then, to test which OTUs were differentially represented in each pre-defined group, we compared benthic and limnetic fish both pooled between lakes and within each lake, using Metastats [35]. We then used SGoF+ [36] to control for the false discovery rate. OTUs were deemed significant at the 0.01 false discovery rate using the above-mentioned procedure. Finally, the consensus sequence of each OTU that was statistically different between each group was assigned to the lowest possible taxonomic level using a BLASTn similarity search against the NCBI 16S ribosomal RNA sequence database. In the cases where the taxonomic assignment did not reach the genus level, we also identified the genera with best-hit BLAST scores.

Ethics statement

The work described here has been conducted according to German law on animal welfare and specifically approved by the Regierungspräsidium Freiburg, Abteilung Landwirtschaft, Ländlicher Raum, Veterinär- und Lebensmittelwesen.

Results and Discussion

Massive parallel sequencing provides an unprecedented opportunity to define the bacterial types that are broadly shared among Midas cichlid fish. We analyzed the sequence variation of 3,347,172 complete 16S rRNA V4 regions (an average of 278,931 sequences per individual – standard deviation: 47,399;
| Cluster | Phylum    | Class            | Order            | Family           | Genus            | Best hit(s)                        | Accession | Abundance (%) |
|---------|-----------|------------------|------------------|------------------|------------------|------------------------------------|-----------|---------------|
| Cluster6060 | Proteobacteria | Gammaproteobacteria | Oceanospirillales | Halomonadaceae | Halomonas | *Halomonas salinifodinae* strain BC7 | NR_044263.1 | 52.8428333  |
|         |           |                  |                  |                  |                   | *Halomonas saccharovorans* strain AJ275 | NR_044117.1 |               |
|         |           |                  |                  |                  |                   | *Halomonas denitrificans* strain M29 | NR_042491.1 |               |
|         |           |                  |                  |                  |                   | *Halomonas kenyensis* strain AIR-2 | NR_043299.1 |               |
|         |           |                  |                  |                  |                   | *Halomonas campaniensis* strain SAG | NR_042157.1 |               |
| Cluster8707 | Proteobacteria | Gammaproteobacteria | Alteromonadales | Shewanellaceae | Shewanella | *Shewanella halisios* strain DW01 | NR_044134.1 | 14.1640167   |
|         |           |                  |                  |                  |                   | *Shewanella algae* strain OK-1 | NR_028673.1 |               |
| Cluster8392 | Firmicutes | Clostridia | Clostridiales | Clostridiaceae | Clostridium | *Clostridium perfringens* strain 13 | NR_074482.1 | 8.269458333  |
|         |           |                  |                  |                  |                   | *Sarcina maxima* strain DSM 316 | NR_026147.1 |               |
|         |           |                  |                  |                  |                   | *Sarcina ventriculi* strain DSM 286 | NR_026146.1 |               |
| Cluster4535 | Proteobacteria | Gammaproteobacteria | Enterobacteriales | Enterobacteriaceae | Citrobacter | *Citrobacter freundii* strain DSM 30039 | NR_028894.1 | 1.730133333  |
|         |           |                  |                  |                  |                   | *Raoultella terrigena* strain 84 | NR_037085.1 |               |
|         |           |                  |                  |                  |                   | *Citrobacter werkmanii* strain CDC 0876-58 | NR_024862.1 |               |
|         |           |                  |                  |                  |                   | *Citrobacter murliniae* strain CDC 2970-59 | NR_028688.1 |               |
|         |           |                  |                  |                  |                   | *Citrobacter braakii* strain 167 | NR_028687.1 |               |
|         |           |                  |                  |                  |                   | *Citrobacter gillenii* strain CDC | NR_041697.1 |               |
| Cluster8049 | Proteobacteria | Betaproteobacteria | Burkholderiales | Comamonadaceae | Comamonas | *Comamonas testosteroni* CNB-2 strain CNB-1 | NR_102841.1 | 1.00715       |
|         |           |                  |                  |                  |                   | *Comamonas testosteroni* strain KC 0043 | NR_029161.1 |               |
| Total   |           |                  |                  |                  |                   |                                |           | 77.45504167   |

doi:10.1371/journal.pone.0095027.t001
Halomonas

doi:10.1371/journal.pone.0095027.g003

respectively – for each sample used in this study.

cordinate axes – explaining 25.72% and 18.67% of variance,

Figure 3. Scatterplot of the scores along the first two principal coordinate axes – explaining 25.72% and 18.67% of variance, respectively – for each sample used in this study.
doi:10.1371/journal.pone.0095027.g003

complete sequencing statistics in Table S1) and identified members of the Gamma- proteobacteria class (genus Halomonas 52% and also Shecavella 14% of total reads) as the most common members of the gut microbiota of adult Midas cichlid fish (see Fig. 2 and Table 1). Even when analyzed at the individual level, the clusters corresponding to these two genera are present in all our samples and constitute a large proportion of their gut microbiota. In fact, Cluster6060 (assigned to the genus Halomonas, see also Table 1) ranges between 31.3% (an A. amarillo individual) and 69.9% (an A. zaliosus individual) of total reads at the individual level. Cluster6707 (assigned to the genus Shecavella; Table 1) makes up between 8.4% (in an A. astorquii specimen) and 18.8% (in an A. zaliosus specimen) of the total reads at the individual level. The same bacterial phyla (predominantly Proteobacteria, but also Firmicutes) were found in gut bacterial communities of other teleost fish in both culture-independent and culture-based surveys [19,37–44]. This commonality of phylotypes suggests that fish harbor bacteria that are typical of the fish gut environment – despite large evolutionary and geographic distances between their fish hosts – rather than reflecting communities from their surrounding environment [19,45].

We found one major difference in the composition of the microbiota of Midas cichlids guts relative to what has been found in other teleost species [19]: members of the Family Halomonadaceae (Order Oceanospirillales) were the most common bacteria. This order was absent in a recent fish bacteria meta-analysis of teleost gut communities based on 19 different species [19]. Generally this order is reported to be halotolerant or halophilic, i.e. adapted to or living in conditions of high salinity, and constitute a large proportion of their gut microbiota. In fact, even when analyzed at the individual level, the clusters corresponding to these two genera are present in all our samples and constitute a large proportion of their gut microbiota. In fact, Cluster6060 (assigned to the genus Halomonas, see also Table 1) ranges between 31.3% (an A. amarillo individual) and 69.9% (an A. zaliosus individual) of total reads at the individual level. Cluster6707 (assigned to the genus Shecavella; Table 1) makes up between 8.4% (in an A. astorquii specimen) and 18.8% (in an A. zaliosus specimen) of the total reads at the individual level. The same bacterial phyla (predominantly Proteobacteria, but also Firmicutes) were found in gut bacterial communities of other teleost fish in both culture-independent and culture-based surveys [19,37–44]. This commonality of phylotypes suggests that fish harbor bacteria that are typical of the fish gut environment – despite large evolutionary and geographic distances between their fish hosts – rather than reflecting communities from their surrounding environment [19,45].

We found one major difference in the composition of the microbiota of Midas cichlids guts relative to what has been found in other teleost species [19]: members of the Family Halomonadaceae (Order Oceanospirillales) were the most common bacteria. This order was absent in a recent fish bacteria meta-analysis of teleost gut communities based on 19 different species [19]. Generally this order is reported to be halotolerant or halophilic, i.e. adapted to or living in conditions of high salinity, and aerobically heterotrophic [41,46,47], and members of the Halomonadaceae are almost exclusively halophilic [47]. In this context it is worth mentioning that the water in the Nicaraguan crater lakes, and in particular in Lakes Apoyo and Xiloá, is not potable and has a high concentration of dissolved salts when compared to the great lakes, Nicaragua and Managua [48].

Further analyses are required to determine the function of these bacteria, and whether these bacteria perform similar functions to other Proteobacteria found in other fish and vertebrate hosts. Whole-genome comparisons of Bacteroidetes species inhabiting the gut, for example, show that their proteomes have similar functional profiles despite differing in 16S rRNA pairwise identity by as much as 12% [49,50]. Reciprocal transplants, for example between zebrfish and Midas cichlid gut microbiota, would allow us to examine whether the cichlid gut environment selects or constrains which members of the microbial populations will dominate and persist – as illustrated by Raads et al. [49] who seeded germ-free zebra fish guts with gut flora from mice.

Do ecotypes differ in their microbiota communities?

The benthic-limnetic axis of adaptive differentiation clearly is important in many freshwater systems. Divergent benthic and limnetic forms have been described in many freshwater fishes including sticklebacks [51–54], whitefish and other salmonids [55–60], perch [61,62], Neotropical Midas cichlids [63,64] and African cichlids [65]. The investigation of the genetic basis of this axis of species differentiation and ecological adaptation has been undertaken with several approaches [37,66–69]. In general, studies of species differentiation have centered on the genetics of habitat specificity [6,70–72]. However, recent evidence suggests that bacterial symbionts may play a key role in resource exploitation and specificity, as well [6]. Clearly, there is also a need to analyze the associations that constitute so-called “metaorganisms” [1]. The two-dimensional representation provided by the principal coordinate plot (Fig. 3) shows a certain degree of separation between different species. In particular, within each lake, limnetic species (A. sagittae and A. zaliosus) show higher scores on the second principal coordinate axis relative to their benthic counterparts.

We explicitly tested whether benthic and limnetic Midas cichlids, which are thought to have arisen rapidly in crater lakes through ecological disruptive selection causing sympatric speciation [23,24], are characterized by different microbial communities using a permutation test with weighted UniFrac distances – i.e. distances between environmental samples which take into account the phylogenetic relationships among bacterial OTUs within each environmental sample [34]. Because the fish used in this study were reared under common environments, any shifts in microbial community would be expected to represent differences in the core characteristic gut microbes rather than subsequent colonization of the hosts by environmental bacteria.

Our analyses show that even under a common rearing environment, significant differences in the microbiota of limnetic and benthic adult cichlid fish are found (weighted UniFrac test, p = 0.04). Within lakes, the comparisons between benthic and limnetic species produced different results. The species pair inhabiting the older crater Lake Apoyo harbors microbial gut communities that are significantly different (weighted UniFrac distance 0.47, p = 0.03). On the other hand, the gut microbiota of the younger crater Lake Xiloá species pair do not differ significantly (weighted UniFrac distance 0.46, p = 0.08). This result is mirrored by the scatterplot of the scores along the first two principal coordinate axes (Fig. 3) as the clusters of the two species from Lake Apoyo (A. astorquii and A. zaliosus) do not overlap. On the other hand, the clusters of the two species from Lake Xiloá, while distinct, show a certain degree of overlap. Interestingly, the weighted UniFrac distances between limnetic and benthic fish from different lakes (0.42 and 0.23, respectively) are lower than the distances between ecomorphs within lakes. The lack of differentiation between the gut communities of the Xiloá species might be a consequence of the lake’s younger age (maximum of 6100 years,[73]) in comparison to Lake Apoyo (with a maximum of 24000 years,[73]), and therefore also younger age of the Midas
Microbial Community Analysis of Cichlid Fish

How do benthic and limnic gut communities differ?
To identify more precisely gut bacterial taxa that differ between *A. astorquii* (benthic) and *A. zaliosus* (limnic) we used a bottom up approach and performed a taxonomical assignment of sequences where read numbers differed between ecomorphs. *A. astorquii* and *A. zaliosus* were different in the relative frequencies of clusters assigned to the genera *Halomonas*, *Shewanella*, *Comamonas*, *Enhydrobacter*, *Vibrio* (see Table S2). *A. zaliosus* had higher relative sequence counts in most clusters whose abundances were significantly different between the two species. In particular, the clusters identified as belonging to the genera *Shewanella* and *Vibrio* had always higher abundance in *A. zaliosus*. On the other hand, *A. astorquii* had significantly higher abundance for clusters assigned to the genera *Enhydrobacter* and *Comamonas*. Further investigations are required to test for the possible roles of these different cichlid fish gut bacteria, and the general mechanisms of symbiosis and gut community assembly. However, the taxonomic differences identified here represent the first candidate symbionts that might be involved in the maintenance and possibly even the origin of different cichlid species that exploit different ecological niches in individual crater lakes.

Supporting Information
Table S1 Sequencing statistics. For each individual, number of sequences before and after the processing steps are shown. (XLSX)

Table S2 For each of the 25 OTUs differentially represented in the comparison between benthic (*A. astorquii*) and limnic (*A. zaliosus*) species of crater Lake Apoyo, sequence similarity search (BLASTn) results are reported. (XLSX)

Author Contributions
Conceived and designed the experiments: PF CF J CJ. Performed the experiments: PF CF J CJ. Analyzed the data: PF CF TF. Contributed reagents/materials/analysis tools: CF J CJ AM. Wrote the paper: PF CF TF J CJ AM.

References
1. Bosch TC, McFall-Ngai MJ (2011) Metaorganisms as the new frontier. Zoology 114: 185–192.
2. Ravil JJ, Samuel BS, Gordon JJ (2004) Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. Proceedings of the National Academy of Sciences of the United States of America 101: 4596–4601.
3. Chenoweth SC, Neal JT, Minty E, Sherlick BM, Guillon K (2011) Epithelial cell proliferation in the developing zebrafish intestine is regulated by the Wnt pathway and microbial signaling via MyD88. Proceedings of the National Academies of Sciences 108: 4570–4577.
4. Moran NA (2006) Symbiosis. Current Biology 16: R866–R871.
5. Douglas AE (2010) The symbiotic habit: Princeton University Press.
6. Brucker RM, Bordenstein SR (2012) Speciation by symbiosis. Trends in ecology & evolution 27: 443–451.
7. McFall-Ngai M, Hadfield MG, Bosch TCG, Carey HV, Domazet-Lošo T, et al. (2013) Animals in a bacterial world, a new imperative for the life sciences. Proceedings of the National Academy of Sciences 110: 3299–3326.
8. Hooper LV, Littman DR, Mcpherson AJ (2012) Interactions Between the Microbiota and the Immune System. Science 336: 1269–1273.
9. Nicholson JK, Holmes E, Kioruno J, Bercelin R, Gibson G, et al. (2012) Host Gut Microbiota Metabolic Interactions. Science 336: 1262–1267.
10. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Marfisi ER, et al. (2006) An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444: 1027–1111.
11. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, et al. (2004) The gut microbiota as an environmental factor that regulates fat storage. Proceedings of the National Academy of Sciences of the United States of America 101: 15718–15723.
12. Heijts RD, Wang S, Anuar F, Qian Y, Björkholm B, et al. (2011) Normal gut microbiota modulates brain development and behavior. Proceedings of the National Academy of Sciences 108: 3047–3052.
13. Li W, Doud SE, Scullock B, Acosta-Martinez V, Lyte M (2009) Memory and learning behavior in mice via the vagus nerve. Proceedings of the National Academy of Sciences of the United States of America 106: 1857–1862.
14. Johnson NA (2010) Hybrid incompatibility genes: remnants of a genomic battlefield? Trends in Genetics 26: 317–323.
15. Sullam KE, Eisinger SD, Louzoupe CA, O’Connor MP, Rosen GL, et al. (2012) Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. Molecular Ecology 21: 3363–3378.
16. Meyer A (1993) Phylogenetic relationships and evolutionary processes in East African cichlid fishes. Trends in Ecology & Evolution 8: 279–284.
17. Kocher TD (2004) Adaptive evolution and explosive speciation: the cichlid fish model. Nature Reviews Genetics 5: 280–298.
18. Wilson AB, Noack-Kunnmann K, Meyer A (2000) Incipient speciation in sympatric Nicaraguan cichlid lake cichlid fishes: sexual selection versus ecological diversification. Proceedings of the Royal Society of London Series B: Biological Sciences 267: 2133–2141.
19. Barbosa M, Stößing KN, Salbarger W, Muschick M, Meyer A (2006) Sympatric speciation in Nicaraguan crater lake cichlid fish. Nature 439: 719–723.
20. Elmer KR, Kusche H, Lehtonen TK, Meyer A (2010) Local variation and parallel evolution: morphological and genetic diversity across a species complex of neotropical crater lake cichlid fishes. Philosophical Transactions of the Royal Society B: Biological Sciences 365: 1763–1782.
21. Recknagel H, Kusche H, Elmer KR, Meyer A (2013) Two new endemic species in the Midas cichlid species complex from Nicaraguan crater lakes: Amphilius tolteca and Amphilius viridis (Perciformes, Cichlidae). aqua, International Journal of Ichthyology 19: 207–224.
22. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, et al. (2012) Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6: 1621–1624.
23. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, et al. (2011) Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences 108: 4546–4552.
24. Williams EE, Rand AS (1977) Species Recognition, Dewlap Function and Faunal Size. American Zoologist 17: 261–270.
25. Kautt AF, Elmer KR, Meyer A (2012) Genomic signatures of divergent selection and speciation patterns in a natural experiment, the young parallel radiations of Nicaraguan crater lake cichlid fishes. Molecular Ecology 21: 4770–4786.
26. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, et al. (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Meth 7: 335–336.
27. Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26: 2460–2461.
28. Meyer F, Paarmann D, D’Souza M, Olson R, Glass E, et al. (2008) The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics 9: 386.
29. Huson DH, Auch AF, Qi J, Schuster SC (2007) MEGAN analysis of metagenomic data. Genome Research 17: 377–386.
34. Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. Applied and environmental microbiology 71: 8228–8235.

35. White JR, Nagarajan N, Pop M (2009) Statistical Methods for Detecting Differentially Abundant Features in Clinical Metagenomic Samples. PLoS Comput Biol 5: e1000352.

36. Carvajal-Rodriguez A, de Uña-Alvarez J (2011) Assessing Significance in High-Throughput Experiments by Sequential Goodness of Fit and q-Value Estimation. PLoS ONE 6: e24790.

37. Huber I, Spanggaard B, Appel K, Rosen L, Nielsen T, et al. (2004) Phylogenetic analysis and in situ identification of the intestinal microbial community of rainbow trout (Oncorhynchus mykiss, Walbaum). Journal of Applied Microbiology 96: 117–132.

38. Navarrete P, Espejo R, Romero J (2009) Molecular analysis of microbiota along the digestive tract of juvenile Atlantic salmon (Salmo salar L.). Microbial ecology 57: 550–561.

39. Navarrete P, Mague F, Mardones P, Riveros M, Opazo R, et al. (2010) Molecular analysis of intestinal microbiota of rainbow trout (Oncorhynchus mykiss). FEMS microbiology ecology 71: 148–156.

40. Romero J, Navarrete P (2006) 16S rDNA-based analysis of dominant bacterial populations associated with early life stages of coho salmon (Oncorhynchus kisutch). Microbial ecology 51: 422–430.

41. Kim H, Choo Y-J, Cho J-C (2007) Litoricolaceae fam. nov., to include Litoricola lipolytica gen. nov., sp. nov., a marine bacterium belonging to the order Oceanospirillales. International journal of systematic and evolutionary microbiology 57: 1793–1798.

42. Tsuchiya C, Sakata T, Sugita H (2008) Novel ecological niche of Cetobacterium somersee, an anaerobic bacterium in the intestinal tracts of freshwater fish. Letters in applied microbiology 46: 43–48.

43. Merrifield DL, Burnard D, Bradley G, Davies SJ, Baker R (2009) Microbial community diversity associated with the intestinal mucosa of farmed rainbow trout (Oncorhynchus mykiss Walbaum). Aquaculture research 40: 1064–1072.

44. Nayak SK (2010) Role of gastrointestinal microbiota in fish. Aquaculture Research 41: 1533–1537.

45. Roosenber G, Mitre EC, Stephens WZ, Parchy DM, Cavanaugh CM, et al. (2011) Evidence for a core gut microbiota in the zebrasid. The ISME journal 5: 1595–1608.

46. Araldr DR, Ventosa A (2006) The family Halomonomadaceae. The prokaryotes: Springer. pp. 811–833.

47. Orben A (2008) Microbial life at high salt concentrations: phylogenetic and metabolic diversity. Salina Systems 4: 13.

48. Barlow GW, Baylis JR, Roberts D (1976) Chemical analyses of some crater lakes in relation to adjacent Lake Nicaragua. Geology 4: 107–121.

49. Rawls JF, Mahowald MA, Ley RE, Gordon J (2006) Reciprocal Gut Microbiota Transplants from Zebras and Mice to Germ-free Recipients Reveal Host Habitat Selection. Cell 127: 423–433.

50. Xu J, Bjarsell MK, Himrod J, Deng S, Carmichael KL, et al. (2003) A genomic view of the human-Bacteroides thetaiotaomicron symbiosis. Science 299: 2074–2076.

51. Larson GL (1976) Social behavior and feeding ability of two phenotypes of Gasterosteus aculeatus in relation to their spatial and trophic segregation in a temperate lake. Canadian Journal of Zoology 54: 107–121.

52. McPhail J (1984) Ecology and evolution of sympatric sticklebacks (Gasterosteus): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. Canadian journal of zoology 62: 1402–1408.

53. Wootten RJ (2009) The Darwinian stickleback Gasterosteus aculeatus: a history of evolutionary studies*. Biological Journal of the Linnean Society 101: 593–608.

54. Willacker JJ, Von Hippel FA, Wilton PR, Walton KM (2010) Classification of three spine stickleback along the benthic-limnetic axis. Biological Journal of the Linnean Society 101: 593–608.

55. Fenderson OC (1964) Evidence of subpopulations of lake whitefish, Coregonus clupeaformis, involving a dwarfed form. Transactions of the American Fisheries Society 93: 77–94.

56. Bernatchez L, Chouinard A, Lu G (1999) Integrating molecular genetics and ecology in studies of adaptive radiation: whitefish, Coregonus sp., as a case study. Biological Journal of the Linnean Society 68: 173–194.

57. Bernatchez L, Renaud S, Whiteley AR, Derome N, Jeukens J, et al. (2010) On the origin of species: insights from the ecological genetics of lake whitefish. Philosophical Transactions of the Royal Society B: Biological Sciences 365: 1783–1800.

58. Landry L, Vincent W, Bernatchez L (2007) Parallel evolution of lake whitefish dwarf ecotypes in association with limnological features of their adaptive landscape. Journal of evolutionary biology 20: 971–984.

59. Bertrand M, Marcogliese D, Magan P (2008) Trophic polymorphism in brook trout revealed by diet, parasites and morphometrics. Journal of Fish Biology 72: 552–572.

60. Stewartson A, Kümmerl R, Adams CE, Przebel K, Amundsen PA (2013) Parallel and non-parallel morphological divergence among foraging specialists in European whitefish (Coregonus lavaretus). Ecology and Evolution.

61. Svanback R, Eklov P (2002) Effects of habitat and food resources on morphology and ontogenetic growth trajectories in perch. Oecologia 131: 61–70.

62. Kekäläinen J, Kalkkinen N, Kirisimäki V, Haankonen H (2010) Morphological variation of perch Perca fluviatilis in humic lakes: the effect of predator density, competition and prey abundance. Journal of Fish Biology 76: 787–799.

63. Meyer A (1990) Ecological and evolutionary consequences of the trophic polymorphism in Cichlasoma ministernum (Pisces: Cichlidae). Biological Journal of the Linnean Society 39: 279–299.

64. Meyer A (1990) Morphometrics and allozymes in the trophically polymorphic cichlid fish, Cichlasoma ministernum: alternative adaptations and ontogenetic changes in shape. Journal of Zoology 221: 237–260.

65. Hulse C, Roberts R, Loh YH, Rupp M, Streelman J (2013) Lake Malawi cichlid evolution along a benthic-­limnetic axis. Ecology and Evolution.

66. Peichel CL, Nereng KS, Oghu GA, Cole BLE, Coloso MF, et al. (2001) The genetic architecture of divergence between threespine stickleback species. Nature 414: 901–905.

67. Rogers SM, Bernatchez L (2005) Integrating QTL mapping and genome scans towards the characterization of candidate loci under parallel selection in the lake whitefish (Coregonus clupeaformis). Mol Ecol 14: 351–361.

68. Albert AY, Sawaya S, Vines TH, Knecht AK, Miller GT, et al. (2008) The genetics of adaptive shape shift in stickleback: pleiotropy and effect size. Evolution 62: 76–85.

69. Franchini P, Fraciano C, Spreiter ML, Jones JC, Elmer KR, et al. (in press) Genomic architecture of ecologically divergent body shape in a pair of sympatric crater lake cichlid fishes. Molecular Ecology doi 10.1111/mec.12590.

70. Hawkinson DJ, Via S (2001) Genetic linkage of ecological specialization and reproductive isolation in pea aphids. Nature 412: 904–907.

71. Naishit RE, Jiggins CD, Mallet J (2003) Mimicry: developmental genes that contribute to speciation. Evolution & development 5: 269–280.

72. Michel AP, Sim S, Powell THQ, Taylor MS, Nosil P, et al. (2010) Widespread genomic divergence during sympatric speciation. Proceedings of the National Academy of Sciences 107: 9724–9729.

73. Kutterolf S, Freundt A, Pérez W, Wehrmann H, Schmincke H-U (2007) Late Pleistocene to Holocene temporal succession and magnitudes of highly-explosive volcanic eruptions in west-central Nicaragua. Journal of Volcanology and Geothermal Research 163: 55–82.

74. Brucker RM, Bordenstein SR (2013) The hologenomic basis of speciation: Gut bacteria cause hybrid lethality in the genus Nausonia. Science 341: 667–669.