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Preliminary observations on the microbiome of *Trachemys scripta scripta* using affordable 16S technologies

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Abstract

Quantifying the microbiome of amphibians and reptiles has the potential to elucidate health, population survival, and ecological dynamics of species in aquatic habitats. Emerging, affordable technologies can provide researchers with baseline data on the microbiome (primarily bacterial communities) of herpeto fauna, an area receiving increasing study. The microbiome of a common freshwater turtle species, *Trachemys scripta scripta*, was evaluated using an affordable 16 S microbiome kit, u Biome Explorer™. This method successfully reported high species diversity identifications from a single turtle fecal sample, with comparable relative percentage of specific taxonomic groupings primarily from the phyla Bacteriodetes, Firmucutes, and Proteobacteria from 127 genera. Researchers should consider collecting baseline microbiome data from herpeto fauna species for conservation and ecological studies.

Keywords: reptile microbiology, turtle ecology, microbiota, yellow-bellied slider turtle

1. Introduction

The vast assortment of microorganisms (microbiome) of herpetofauna remain a vastly unexplored, emerging area of scientific research. The gut microbiome of vertebrates, including amphibians and reptiles, has a vital role to their host organisms by contributing to health, development, and host immunity (Bletz *et al*., 2013, Lee and Hase, 2014) [3, 14], and is an important component to the survival of herbivorous vertebrates (Williams *et al.*, 2001) [22]. Given the often beneficial symbiotic relationship many taxa possess with these microorganisms, research into the vertebrate microbiome may hold the potential for new discoveries related to emerging infectious diseases and other insights, as the number of microbial organisms may outnumber the number of host cells in many vertebrates (Karsov *et al.*, 2011, McKenzie *et al*., 2012) [12, 15]. While several studies have focused on a variety of reptiles, primarily marine turtles (Ahasan *et al.*, 2017) [2], alligators (Keenan and Elsey, 2015) [13], and lizards (Hong *et al*., 2011) [8], in comparison freshwater turtles have received far less attention. Research on the microbiome of marine turtles has largely outpaced research on freshwater turtles, and has identified several groups of microbiota (Price *et al.*, 2017, Campos *et al.*, 2018) [18, 4]. However, other than a limited number of studies on captive individuals (Ruzauskas *et al.*, 2016) [19], assessment of freshwater turtle Micro biomes is currently lacking. As aquatic turtles likely harbor a great wealth of unique associations with bacteria as do other vertebrates, including those microorganisms associated with disease or host immunity, metabolism, or her bivory (Colston and Jackson, 2016) [5], there remains much promise for future discovery of diversity in the reptile microbiome by utilizing more recent affordable genomic technologies.

Next-generation sequencing provides an emerging avenue for the study of reptile microorganisms, albeit with an associated cost for large numbers of samples potentially being cost prohibitive (~ $3,000-5,000 US or more), depending on project parameters, number of samples, and genomic tools utilized by researchers. While the cost per sample is diminishing (Goodwin *et al*., 2016) [7], many researchers may lack either funding or the necessary laboratory and data analysis experience to perform preliminary study of a specific amphibian or reptile species from data generated by genomic studies. Application of standard technologies, such as next generation 16S ribosomal RNA sequencing, provides an affordable method to identify and enumerate relative abundance of microbial communities. Moreover, these increasingly affordable technologies allow for researchers studying herpeto fauna of remote areas to contribute to the study of microbiome, as little is required for field collection (sterile swabbing and sample container) and can potentially allow comparisons of the microbiome across taxonomic groups and for researchers with limited funding and experience studying populations of species of conservation concern characterized by small sample sizes.

The yellow-bellied slider, *Trachemys scripta scripta*, (Testudines: Emydidae) is a common turtle found throughout the southeastern United States (Powell *et al*., 2016) [17]. This short communication is among the first to characterize a freshwater turtle fecal bacterial community (microbiome) utilizing emerging, affordable technologies (~ $100). This study, while limited to a single *Trachemys scripta scripta* (n = 1), provides baseline information on taxonomic diversity housed in the microbiome of wild freshwater turtles, and reports on the feasibility of a readily available, affordable citizen science uBiome Explorer™ kit as a method to characterize gut microbial community diversity in reptiles. References should be like this [1, 2, 3].

2. Materials and methods

An adult female yellow-bellied slider, *Trachemys scripta scripta* (Thunberg, 1792) (Testudines: Emydidae) (mass = 380 g, straight carapace length = 150 mm), was collected on September 28, 2018, as part of ongoing mark-recapture studies at Campus Lake, a small pond located on the campus of Wingate University, Wingate, North Carolina, United States (“34° 59’12.43 N, 80°25’47.59” W). To minimize contamination of fecal material the turtle was kept under observation following capture in a pre-cleaned, disinfected plastic container and monitored for 4 hours until a fecal sample was collected. Upon observation of defecation, the fecal pellet was immediately removed from the container (~15 seconds following defecation by individual turtle) using sterile gloves, and weighed to the nearest 0.1 gram. The sample size consisted of a 2.8 g fecal sample (wet mass). Following collection of the fecal pellet the individual was released at the study site. Fecal material was selected over cloacal sampling, as it may provide a greater representation of the bacterial communities of the colon (Videvall *et al.*, 2017) [21].

To process the sample, I followed the uBiome Explorer™ kit instructions for collection of sample. In brief, the sample was placed carefully on provided tissue, and a sterile swab was run lightly over the sample fully covering the swab, and inserted into the provided 1.5 µl tube containing proprietary buffer. The swab was stirred and shaken for one minute and the swab was removed. Next the sample tube was sealed and sent to uBiome (https://www.ubiome.com) in the provided sealed return mailer bag. U Biome is a private citizen science microbial genomics laboratory which utilizes 16S rRNA gene precision sequencing™ and provides customers with raw data on microbial diversity down to the genus and species level, following the Human Microbiome Project Consortium, 2012. The sample kit was activated and registered online, and sequence data was available within two months. An excel sheet with raw sequence data was downloaded on 12/15/18, and dominant taxa (phyla and class) were examined.

3. Results and Discussion

In total 16 phyla, 30 classes, 56 orders, 89 families, 127 genera, and 89 putative species of microorganisms were identified using 16S rRNA sequencing from uBiome (Appendix I). A further 14 organisms were assigned no rank and were deemed present, but taxonomically unclassified. The microbiome of the *T. scripta scripta* individual consisted primarily of the following dominant phyla: Bacteroidetes (59.9%), Firmicutes (31.9%), Proteobacteria (3.9%), Lentisphaerae (1.7%), and Verrucomicrobia (1.5%), with remaining phyla comprising less than 1% each of fecal pellet, represented by Fusobacteria, Actinobacteria, Cyanobacteria, Planctomycetes, Terenicutes (Fig. 1). Moreover, fecal sample was dominiated by the bacterial classes Bacteroidia,

Clostridia, and Flavobacteriia, which comprised the highest relative abundance (Fig. 2). According to this data, Bacteriodetes and Firmicutes followed by Proteobacteria account for the majority of bacteria, which is similar to findings of Abdelrhman *et al.*, 2016 [1] for *Caretta caretta* and also Ahasan *et al.*, 2017 [2] for *Chelonia mydas*, for the top three most represented bacterial phyla in the microbiome of sea turtles. However, Price *et al.*, (2017) [18], found higher overall percentages for microorganisms from Proteobacteria in juvenile green turtles, *Chelonia mydas*. Studies of other reptiles in addition to the limited scientific research on sea turtles, have found the gut microbiome to be dominated by Proteobacteria and Bacteroidetes as in alligator lizards (Jiang *et al.* 2017) [11] and Fusobacteria and Clostridia in wild and farm raised American Alligator *Alligator mississippiensis* alligators (Keenan and Elsey, 2015) [13]. Therefore, the results of this present study appear to be comparable to other findings for commonly encountered bacterial communities at the phyla level which may be important for bacterial gut health in herbivorous reptiles.

Consequently, at the organismal level of bacterial class, this present study found many of the same classes as both Price *et al.* (2017) [18] and Abdelrhman *et al.*, (2016) [1], albeit at different relative abundances (Fig. 2). Interestingly, Clostridia, the second most predominant class of bacteria identified in this study, was also reported as a commonly occurring bacterial class in both wild and farm-raised freshwater American alligators, *Alligator mississippiensis* (Keenen and Elsey, 2015) [13]. In addition, several dominant bacteria genera identified included *Flavobacterium*, *Macelli bacteroides*, *Sarcina*, and *Para bacteroides* (Appendix 1), which may have roles as herbivorous gut associated microbes, resistant inhibitors of other bacteria with potential antimicrobial properties, or opportunistic pathogens in freshwater turtles (Jacobson *et al.*, 1989, Hong *et al.*, 2011, Nakano *et al.*, 2011, Ahasan *et al.*, 2017) [10, 8, 16, 2].

Freshwater turtle diet, including that of *Trachemys scripta scripta*, may vary across populations, habitats, and age classes, but includes likely a combination of animal and plant matter (Dreslik, 1999) [6], which requires maintaining micro flora for proper digestion and microbial fermentation. While more advanced sequencing is available and this research was limited to one individual, the future use of affordable microbiome kits appears promising for preliminary studies on herpetological species. However, researchers can more fully explore any microbiome data obtained from uBiome or other companies (i.e., American Gut) using readily available software (Sprague, 2015) [20]. Overall, the results of this preliminary survey of a freshwater turtle microbiome is comparable to other studies.

4. Tables and Figures

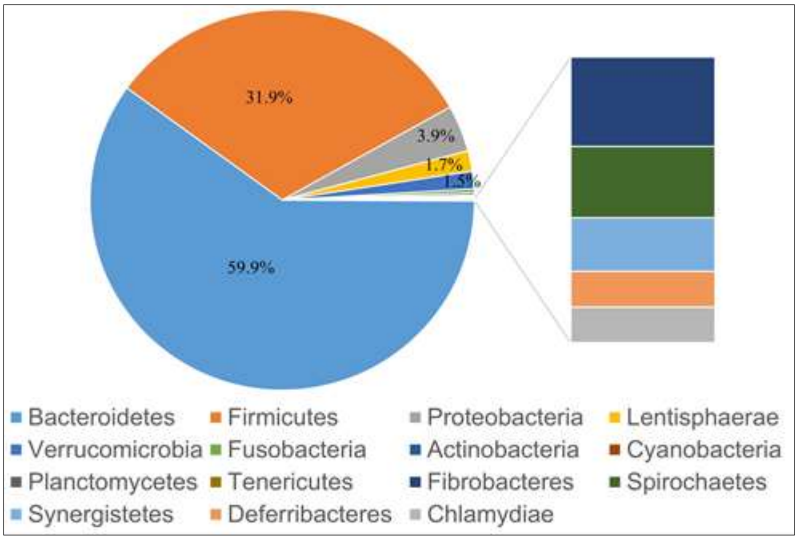

Fig 1: Relative abundance (%) of bacterial community phyla identified for *Trachemys scripta scripta*.

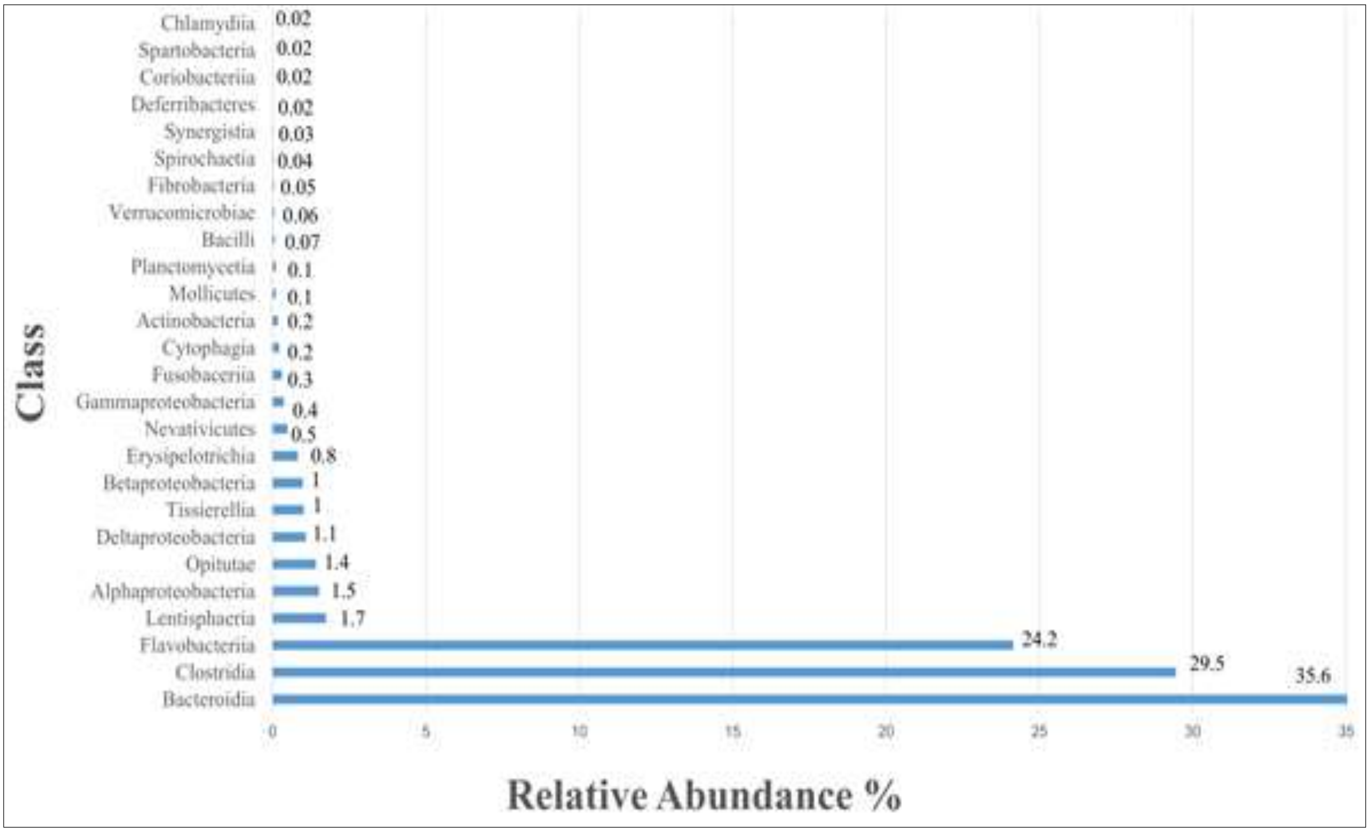

Fig 2: Relative abundance (%) of bacterial community classes for *Trachemys scripta scripta*. Results are shown ranked with the most dominant classes at the bottom of the Y-axis.

5. Conclusions

When these preliminary survey of turtle micro flora are taken collectively, these findings illustrate the potential for herpetologist to conduct preliminary investigations of the microbiome in the amphibian or reptile population of their choice, using an affordable method resulting in efficient preliminary data gathering of microbial community diversity of her peto fauna.

6. Appendix 1

Microbial genera and counts identified via 16S rRNA sequencing for Trachemys scripta scripta single sample in alphabetical order.

Acinetobacter (7), Actinocorallia (2), Aeromonas (17), Alistipes (430), Alsobacter (8), Anaerobacter (6), Anaerobacterium (47), Anaerofilum (16), Anaerofustis (4), Anaerosinus (157), Anaerosporobacter (1635), Anaerotruncus (505), Anaerovorax (201), Aphanizomenon (4), Aurantimonas (7), Azoarcus (6), Azospira (13), Bacillus (10), Bacteroides (390), Bilophila (372), Blautia (679), Bradyrhizobium (639), Butyricicoccus (11), Butyricimonas (11), Candidatus Methanomethy lophilus (36), Candidatus Microthrix (6), Candidatus Rhabdochlamydia (6), Candidatus Soleaferrea (68), Catenibacterium (57), Cellulosilyticum (1518), Cetobacterium (268), Christensenella (2), Cloacibacillus (2), Clostridioides (189), Clostridium (237), Collinsella (14), Coxiella (4), Denitratisoma (264), Desulforhabdus (59), Desulfoto maculum (9), Desulfovibrio (495), Devosia (247), Edwardsiella (4), Epulopiscium (840), Erysipelatoclostridium (2), Eubacterium (15), Exiguo bacterium (3), Faecalibacterium (69), Fibrobacter (40), Flavobacterium (20738), Flavonifractor (701), Fodinicola (4), Fonticella (250), Fulvimarina (4), Geobacter (2), Geodermatophilus (4), Gordonibacter (5), Herbaspirillum (24), Hespellia (557), Hydro genoanaero bacterium (15), Hydro genophaga (7), Hymenobacter (185), Intestinibacter (2976), Intestinimonas (19), Klebsiella (27), Lactonifactor (5), Laribacter (114), Legionella (8), Macelli bacteroides (10770), Methanobacterium (8), Methano brevi bacter (3), Methanocorpusculum (21), Methanothrix (36), Methyl obacterium (9), Methy localdum (6), Methyl ocystis (2), Methyl osinus (15), Methyl otenera (2), Micro bacterium (3), Microcystis (3), Mycobacterium (47), Natranaerovirga (388), Neomegalonema (9), Nitro bacter (11), Nordella (14), Ocean irhabdus (32), Odori bacter (1364), Oscilli bacter (384), Oscillospira (6), Paludi bacter (139), Papilli bacter (7), Para bacteroides (5313), Para sporo bacterium (818), Pepto clostridium (29), Phasco larctobacterium (6), Phocaeicola (7), Plano microbium (18), Prop ionispora (3), Proteiniphilum (41), Pseudaeromonas (43), Pseudobutyrivibrio (280), Rahnella (173), Rhizobium (2), Rhodobacter (288), Rickettsia (3), Rickettsiella (13), Rikenella (2), Robinsoniella (323), Romboutsia (3006), Roseburia (45), Roseococcus (3), Roseomonas (19), Sarcina (6476), Sedimentibacter (153), Singulisphaera (2), Sporobacter (68), Staphylococcus (3), Streptomyces (74), Streptosporangium (5), Subdoligranulum (109), Synechococcus (91), Terrisporobacter (1031), Thalassospira (8), Turicibacter (556), Victivallis (668), Vogesella (386), Zavarzinella (4).

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