SYMPOSIUM

The Good, the Bad, and the Unknown: Microbial Symbioses of the American Alligator

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Synopsis Vertebrates coexist with microorganisms in diverse symbiotic associations that range from beneficial to detrimental to the host. Most research has aimed at deciphering the nature of the composite microbial assemblage’s genome, or microbiome, from the gastrointestinal (GI) tract and skin of mammals (i.e., humans). In mammals, the GI tract’s microbiome aids digestion, enhances uptake of nutrients, and prevents the establishment of pathogenic microorganisms. However, because the GI tract microbiome of the American alligator (Alligator mississippiensis) is distinct from that of all other vertebrates studied to date, being comprised of Fusobacteria in the lower GI tract with lesser abundances of Firmicutes, Proteobacteria, and Bacteroidetes, the function of these assemblages is largely unknown. This review provides a synthesis of our current understanding of the composition of alligators’ microbiomes, highlights the potential role of microbiome members in alligators’ health (the good), and presents a brief summary of microorganisms detrimental to alligators’ health (the bad) including Salmonella spp. and others. Microbial assemblages of the GI tract have co-evolved with their vertebrate host over geologic time, which means that evolutionary hypotheses can be tested using information about the microbiome. For reptiles and amphibians, the number of taxa studied at present is limited, thereby restricting evolutionary insights. Nevertheless, we present a compilation of our current understanding of reptiles’ and amphibians’ microbiomes, and highlight future avenues of research (the unknown). As in humans, composition of microbiome assemblages provides a promising tool for assessing hosts’ health or disease. By further exploring present-day associations between symbiotic microorganisms in the microbiomes of reptiles and amphibians, we can better identify good (beneficial) and bad (detrimental) microorganisms, and unravel the evolutionary history of the acquisition of microbiomes by these poorly-studied vertebrates.

Introduction

Defining symbioses in vertebrates

In the broadest sense, symbioses—positive, negative, or neutral interactions between two organisms—drive life. From nitrogen-fixing bacteria that live in association with plant roots (e.g., Mylona et al. 1995) to wasps that lay eggs in caterpillars (e.g., Whitfield 2002), life is a complex series of symbiotic associations. Vertebrates, including the American alligator (Alligator mississippiensis), are colonized by diverse assemblages of microorganisms in a variety of symbiotic interactions. However, distinguishing what represents a “good” rather than a “bad” association is not straightforward and has recently undergone a transformation. In particular, the revelation that a vertebrate contains an order of magnitude more microbial than eukaryotic cells (e.g., Karasov et al. 2011) has shifted our understanding of the role of microorganisms in hosts’ health or disease (Hooper and Gordon 2001).

Symbiotic associations between a vertebrate and microorganisms occur on every surface from the skin to the gastrointestinal (GI) tract lumen (Grice et al. 2008; Human Microbiome Project Consortium 2012). Often, these interactions are beneficial for the host or have a neutral impact on the host’s health. Relative stability is disrupted through the establishment of pathogenic microbes or alteration to mutually-communities (dysbiosis), negatively impacting
health (e.g., Hooper and Gordon 2001). In alligators, microbially caused diseases (see discussion below) often are accompanied by physical manifestations, such as inflammation or discoloration. However, identifying “good” versus “bad” microbial colonization is not always straightforward (Cho and Blaser 2012), particularly in the GI tract. This review will include a discussion of our current understanding of “good” (beneficial) and “bad” (detrimental) microbial associations in the American alligator. We also highlight future directions for exploring the large unknown or unexplored interactions between a vertebrate and its microorganisms in the context of reptiles and amphibians. Elucidating the nature of these vertebrate–microbe interactions holds promise for identifying symbiotic associations maintained over geologic time as well as assessing the health or disease of the host.

Microenvironments of the GI tract

The GI tract of vertebrates consists of distinct organs and tissues extending from the mouth to the colon. Physiologically, each region of the GI tract plays a specific role in the breakdown and uptake of nutrients (Karasov et al. 2011). Each of these regions presents distinct physicochemical conditions, availability of nutrients, rates of turn-over in the lumen, surface areas for attachment, and thus would be expected to be inhabited by environment-specific and niche-specific microorganisms (Walter and Ley 2011). For example, the pH of the stomach is highly acidic (in alligators, pH ranges from 2.1 to 3.0 during feeding) (Keenan et al. 2013), while other areas like the large intestine have a circum-neutral pH (personal observation). For microorganisms, the diversity of available nutrients as well as surfaces for attachment present constraints on metabolism.

The skin as a microenvironment

The GI tract provides microbial assemblages with relatively stable physicochemical conditions in each distinct region along the digestive system. In some vertebrates, including snakes and alligators, changes in diet or feeding status result in seasonally-variable physicochemical conditions (Costello et al. 2010; Keenan et al. 2013). In contrast, microbial assemblages colonizing the skin of a vertebrate are faced with a distinct suite of physical and chemical conditions. For semi-aquatic vertebrates like crocodilians, the transition from land to water regularly throughout the day exposes microorganisms to widely variable temperature, salinity, UV light, and availability of water and oxygen. The cutaneous microbiome of alligators, and crocodilians in general, is currently unknown. However, given the variability in composition of the microbiome observed in humans (e.g., Grice et al. 2008, 2009), a similar pattern would be expected in alligators. A recent evaluation of the microbial diversity on human skin suggested that biogeographic and niche partitioning, driven both by microenvironment and by the host’s individuality, exerted controls on the resulting bacterial, archaeal, and viral diversity (Oh et al. 2014).

Guided by these insights from humans, skin among the dorsal and ventral surfaces would be expected to contain dissimilar microbial assemblages due to the distinct microenvironments present on these surfaces. Crocodilian skin is thick (epidermal and dermal thickness of 30–150 μm and >250 μm, respectively) (Alibardi 2011) and composed of β-keratin and collagen (Alibardi and Toni 2007; Dalla Valle et al. 2009). Morphologically, dorsal and ventral surfaces are distinct. The dorsal surface is comprised of ridges and troughs, due to the presence of ossified bones or scutes, that form a complex surface. In contrast, the ventral surface is more uniform with thick, flat keratinized scutes. The surface morphology of the ventral and particularly the dorsal surfaces provides a large surface area for the attachment and growth of microbial biofilms.

In addition to being morphologically distinct, the dorsal and ventral surfaces are exposed to variable physicochemical conditions on daily, as well as seasonal, intervals. Dorsal surfaces are regularly exposed to variable UV, water, pH, salinity, and temperature as alligators move from water to land. Ventral surfaces are likely to experience variable availability of water and oxygen, but perhaps not as much exposure to UV. These distinct physical and chemical conditions will exert large controls on composition of the microbiome. Based on what has been observed on the skin of humans, mice (e.g., Grice et al. 2008, 2009), and amphibians (Kueneman et al. 2014), the greatest diversity of microorganisms will likely be members of Proteobacteria with lesser representation from Actinobacteria and Firmicutes.

The good: microorganisms beneficial to alligator health

Microorganisms are widely viewed as harmful or detrimental to an animal, often being associated with disease or illness. However, as novel approaches toward characterizing the microbial world around us have changed, particularly a departure from strictly culture-based approaches (Hugenholtz et al. 1998), microorganisms are now viewed in a different light.
The ability to obtain the genetics of microorganisms within an environmental sample, such as an oral swab, through “next generation sequencing”, has revolutionized our understanding of microbial ecology and diversity. Through these novel techniques, characterizing the microbiome residing within and on an organism is now feasible. In an animal, microbes are not only beneficial for the host’s health by aiding the absorption of nutrients (Karasov et al. 2011), but may also be required for preventing the proliferation of harmful microorganisms (Peralta-Sanchez et al. 2014) and for the development of the host’s immune system (Kau et al. 2011), including allergen sensitization (Stefka et al. 2014).

Establishment of a microbiome

The composition of a microbiome reflects the influence of a variety of interactions between a vertebrate host and its environment, diet (prey items), genetics, and interactions within the microbiome itself (Fig. 1). The relative contribution of each component to the composition is unknown. Some studies demonstrated the influence of geographic location or environment on the composition (e.g., Yatsunenko et al. 2012), suggesting that perhaps these sets of variables exert the greatest control. Other studies, including one of the Burmese python (Costello et al. 2010) highlight the role of diet or feeding status on modulating the composition of microbial assemblages. Additionally, a similar conclusion has been reached by several studies focusing on the microbial ecology of the GI tracts of humans and other mammals (e.g., Nelson et al. 2013). The role of the host’s genetics has also been proposed as a significant modulator of the GI tract. Supporters of the idea that a host’s genetics controls the composition of microbiomes demonstrated the ability to reconstruct hosts’ phylogenetics on the basis of the composition and diversity of their resident microbial consortia (e.g., Ochman et al. 2010). Interactions, including competition among microbes within the gut (e.g., Roggenbuck et al. 2014) and between microbes and the host itself also control membership of the assemblage (e.g., Artis 2008) (Fig. 1).

There are likely to be mixed contributions from these four components—genetics, environment, diet of the host, and interactions among members of the microbiome—that shape a vertebrate’s microbiome (Fig. 1). It is likely that throughout the course of a vertebrate’s life, there are periods when the composition of the microbiome is altered due to aging (e.g., in humans; Heintz and Mair 2014), illness (e.g., Cho and Blaser 2012), or changes to diet (e.g., Turnbaugh et al. 2008). On longer, geologic timescales, the emerging view of the GI tract’s microbiome as co-evolved with the host suggests that membership of the microbial assemblage has undergone a variety of selective pressures resulting in the microbiome observed today (Ley et al. 2008b). Co-evolution between the vertebrate host and its resident microbial assemblages creates strong selective pressures to keep the beneficial microorganisms, and defend against detrimental invaders. While determining if a certain bacterial “species” is beneficial or harmful for the host is still no small feat, a picture of the “core” microbiome for various vertebrates is starting to emerge (Turnbaugh et al. 2007; Caporaso et al. 2011; Keenan et al. 2013).
the oral cavity, the composition of the microbial assemblage not only reflects the endogenous, resident microorganisms but also those acquired through contact with the environment (e.g., sediment, water, and vegetation). In alligators and other crocodilians that employ basking as a method of thermoregulation (Lang 1987; Seebacher et al. 2003), visible green to brown biofilms along the tongue indicate the establishment of environmentally-derived microorganisms (e.g., Proteobacteria and Actinobacteria). At the level of the bacterial class, oral communities in actively feeding alligators are dominated by Clostridia, Fusobacteria, and members of Proteobacteria (Fig. 2A). Members of the same classes are also present in winter, fasting alligators (Keenan et al. 2013).

The stomach presents a microenvironment distinct from that of the rest of the GI tract. Highly acidic pH, as well as an influx of transient, food-derived microorganisms, results in a dynamic system. Assemblages of free-living and attached bacteria were similar, and consisted overwhelmingly of Clostridia (~80% of the assemblage) (Fig. 2B; Keenan et al. 2013). Similarly, in the duodenum, Clostridia are numerically dominant, with lesser contributions from Epsilonproteobacteria and unclassified Bacteria (Fig. 2C).

The intestines have highly folded mucosa, leading to high surface-area:volume ratios that facilitate the uptake of nutrients. These surfaces also provide areas for microbial colonization and the development of biofilms. In the duodenum and ileum, Clostridia and Bacilli dominated the bacterial assemblage. Notably, Fusobacteria, which were prominent in the mouth, as well as in the lower GI tract, formed a relatively minor fraction of the assemblage, representing approximately 2–6%. In the colon as well as in the feces, Fusobacteria become the dominant phylum. In the colon and feces, Clostridia are also significant members of the assemblage. The diversity of tissues and functions along the length of the GI tract, as well as the distinct physiochemical environments, would be expected to result in diverse microbial assemblages, and this is observed along the length of the GI tract, particularly in the less well-represented classes (Dethlefsen et al. 2007; Keenan et al. 2013; Keenan 2014a, 2014b).

Using the insights gained for different bacterial groups in modulating the health or disease of humans, we can begin to piece together likely functional roles for different bacteria in modulating alligators’ health (or disease). Changes in the ratio of Firmicutes and Bacteroidetes, dominant both in humans and in alligators, have been linked to changes in overall health of the host, particularly obesity (Ley et al. 2005; Turnbaugh et al. 2006, 2008, 2009; Keenan et al. 2013). In farm-raised alligators, which contained greater mesenteric fat deposits than their wild counterparts, we noted similar shifts in composition of the microbiomes as observed in humans (Keenan et al. 2013). Specifically, an increase in Firmicutes relative to Bacteroidetes in humans, mice, and alligators, supports a direct link between these two phyla and overall health of the host; however, specific links directly identifying obesity have recently been questioned (e.g., Fincane et al. 2014). The recent discovery of similar Fusobacteria-dominated microbiomes in vultures by Roggenbuck et al. (2014) provided new insights into the role of Fusobacteria in modulating the health of alligators. Roggenbuck et al. (2014) demonstrated that members of Fusobacteria as well as Clostridia in the guts of vultures provided a level of defense to the host, allowing consumption of partially decomposed carrion containing bacterially-produced toxins. The authors suggested that Fusobacteria and Clostridia, dominant members of the microbiome, may be integral to the host’s health.

The microbiome plays a critical role in establishing the host’s immune system, immune responses, and resistance to disease (Turnbaugh et al. 2006). Studies have shown that alligators exhibit a febrile response to infection (Merchant et al. 2007) and have a strong capacity to resist infection. Additionally, their blood has antibacterial (Merchant et al. 2003), amoebicidal (Merchant et al. 2004), and antiviral properties (Merchant et al. 2005). It may be that wild alligators do succumb to microbially-caused malignancies but, after death in the wild, carcasses may rapidly deteriorate in ambient heat and humidity, and are not recovered by researchers. Part of alligators’ exceptional resistance to disease may be due to a tight co-evolution with their gut microbiome, thereby helping to hone their immune response (Merchant et al. 2003, 2006; Keenan et al. 2013). Similar suggestions for a link between development of the immune system and the composition of the microbiome in humans have transformed our understanding of the role of microorganisms in hosts’ health and disease (e.g., O’Hara and Shanahan 2006; Kau et al. 2011).

The bad: detrimental microorganisms associated with alligators

Microbial pathogens affecting alligators

Despite a robust immune system, alligators are susceptible to microbial infection from a variety of pathogens. Gram-negative septicemia has been noted in captive alligators. In one study, six bacterial
Fig. 2 Average abundance of different classes of bacteria from wild and farm-raised alligators, post-feeding. Samples were obtained along the length of the GI tract, as indicated by letters A–F: (A) mouth/esophagus, (B) stomach, (C) duodenum, (D) ileum, (E) colon, (F) feces (data available from Keenan et al. [2013] and the Supplementary Information file described in that paper).
species were implicated in septicemia (family Enterobacteriaceae) and were not previously associated with disease in alligators (Novak and Seigel 1986). There are numerous other bacterial as well as fungal species that have been isolated from infected alligators (Table 1). While far from complete, the list of known pathogens (Table 1) spans many of the phyla that also contain normal members of the microbiome of alligators, including Actinobacteria, Firmicutes, Bacteroidetes, Proteobacteria, and Fusobacteria. Identifying microbially-caused diseases in wild populations is difficult, if not impossible, without subsequent analyses of the sites of infection. Research assessing wild crocodiles' abnormalities and injuries, which have the potential to become infected, are relatively limited. A thorough study of 1345 wild-caught saltwater crocodiles (Crocodylus porosus) only identified three (0.2%) with “growths” (Webb and Messel 1977), possibly former sites of microbial infection. A similar survey of 797 wild-caught freshwater crocodiles (Crocodylus johnstoni) noted only six “growths”, three of which appeared to be sites of microbial infection (Webb and Manolis 1983). A smaller study of diseases of 144 wild-caught Nile crocodiles (Crocodylus niloticus) did not reveal any growths or tumors due to prior microbial infection, and the only external lesions were an old bite and a recent puncture from a bite (Leslie et al. 2011). A review by Huchzermeyer (2003) of transmissible crocodilian diseases (viral, bacterial, fungal, and parasitic) highlights the physical manifestations of microbial infections in crocodilians.

**Microbes associated with alligator bites on humans**

Although rare, alligators can attack or incidentally bite humans, particularly when humans participate in

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<th>Microbial pathogen</th>
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<tr>
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<td>Aspergillus ustus</td>
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<td>Aspergillus fumigatus</td>
<td>Jasmin et al. (1968)</td>
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<td>Scopulariopsis sp.</td>
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<td>Gammaproteobacteria</td>
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<td>Nevarez et al. (unpublished data)</td>
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<tr>
<td>Acinetobacter spp.</td>
<td>Nevarez et al. (unpublished data)</td>
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<td>Aeromonas hydrophila</td>
<td>Novak and Seigel (1986); Sartain and Steele (2009)</td>
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<td>Chryseomonas luteola</td>
<td>Nevarez et al. (unpublished data)</td>
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<td>Citrobacter braakii</td>
<td>Nevarez et al. (unpublished data)</td>
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<td>Citrobacter freundii</td>
<td>Novak and Seigel (1986); Sartain and Steele (2009)</td>
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<td>Edwardsiella tarda</td>
<td>Wallace et al. (1966)</td>
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<td>Enterobacter agglomerans</td>
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<td>Enterobacter cloacae</td>
<td>Nevarez et al. (unpublished data)</td>
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<td>Escherichia coli</td>
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<td>Klebsiella oxygena</td>
<td>Novak and Seigel (1986)</td>
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<td>Morganella morganii</td>
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<td>Proteus vulgaris</td>
<td>Wallace et al. (1966); Sartain and Steele (2009)</td>
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<td>Pseudomonas spp.</td>
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<td>Serratia marcescens</td>
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<td>Enterococcus spp.</td>
<td>Sartain and Steele (2009)</td>
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<td>Mycoplasma alligatoris</td>
<td>Clippinger et al. (2000)</td>
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<td>Staphylococcus spp.</td>
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<td>Fusobacteria</td>
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<td>Fusobacterium varium</td>
<td>Sartain and Steele (2009)</td>
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recreational activities in the water (Conover and Dubow 1997). In addition to the traumatic injuries from alligator bites, victims may suffer infections from various microorganisms, particularly Gram-negative bacteria (Langley 2005). Numerous pathogenic species (aerobic and anaerobic bacteria; fungi) have been cultured from the mouths of crocodilians (Langley 2005). The oral flora of the American alligator has been described, and recommended initial antibiotic therapy for alligator bites has been recommended (Flandry et al. 1989). A wide variety of microorganisms were cultured from alligators and water samples in the study by Flandry et al. (1989) including 20 aerobic and 18 anaerobic bacterial species, and 20 fungal species. The most frequently isolated microorganisms included members of Proteobacteria (*Aeromonas hydrophila*, *Proteus vulgaris*) in addition to *Pseudomonas* spp., *Clostridium* spp., and *Bacteroides* spp. Our work characterizing the oral microbiome of alligators (Fig. 2; Keenan et al. 2013), the work of Flandry et al. (1989) and others (Table 1) captured many of the dominant members of the assemblage in their culture-based work, but missed some of the other dominant taxa, namely Fusobacteria. Sartain and Steele (2009) reported a traumatic amputation of an upper extremity in a youth following an alligator bite, and summarized the literature on microorganisms associated with alligator bites (Table 1).

The unknown: current and future research on microbiomes

The ability to employ culture-independent methods to evaluate microorganisms in environmental samples, including tissues of the GI tract, has revolutionized our view of the GI system and our understanding of health and disease. The application of next-generation sequencing has revealed some striking patterns in the GI tract’s microbial assemblages. Notably, all vertebrate GI tracts studied contain a similar and conservative assemblage of a few bacterial phyla, dominated by Firmicutes, Bacteroidetes, and Proteobacteria, with lesser representation by Fusobacteria, Actinobacteria, and several other phyla (Ley et al. 2008a, 2008b). In some taxa, including mammals (e.g., humans and mice) and reptiles (e.g., alligators), the ratio of Firmicutes to Bacteroidetes has been used as an indicator of obesity or health of the host (Ley et al. 2005; Turnbaugh et al. 2006, 2008, 2009; Keenan et al. 2013).

Elucidating the role of Fusobacteria in the GI tract

At the phylum-level, Fusobacteria are poorly described in terms of environmental distribution, metabolic activities, and even their phylogenetic position on the tree of life (Mira et al. 2004; Battistuzzi and Hedges 2008). One estimate places their origin among the earliest branches on the tree of life around 3.5 billion years ago (Battistuzzi and Hedges 2008), while another suggests a more recent origin around 400 million years ago (Mira et al. 2004), coincident with the evolution of vertebrates and the GI tract.

Part of this uncertainty with respect to phylogenetic placement is due to limited studies of non-pathogenic Fusobacteria, essentially leaving our understanding of genetics, metabolic functioning, and distribution of this phylum incomplete. Fusobacteria have been recovered from a narrow diversity of environmental samples, including the oral cavity of humans (e.g., Bradshaw et al. 1998), liver abscesses, foot rot, oral necrotic lesions, farm soil (Langworth 1977), and several other environmental locations (e.g., ~100-cm-deep snow, Moller et al. 2013). Members of this phylum have largely been studied in the context of pathogens of humans and other animals (e.g., *Fusobacterium necrophorum*) (Langworth 1977; Nagaraja et al. 2005). They are Gram-negative, anaerobic, non-spore-forming, and when isolated from an animal, are considered to be an indication of disease (Langworth 1977; Tan et al. 1996). They often are viewed as opportunistic pathogens, flourishing when the host is physically or immune-compromised by injury or illness (Tan et al. 1996).

The overwhelming majority of research on Fusobacteria relates directly to human oral health. The presence of Fusobacteria is widely associated with the formation of biofilm in the mouth, leading to dental carries and the buildup of plaque (Munson et al. 2004). However, they form a minor component of the gut microbiome of humans, suggesting limited to no colonization of the digestive system other than the oral cavity. In contrast, there is a dominance of Fusobacteria in the lower GI tract of alligators (Keenan et al. 2013; Keenan 2014a, 2014b). More recently, this phylum was found to dominate vulture’s microbiome (Roggenbuck et al. 2014). From the few studies describing Fusobacteria isolated from environmental samples, they are characterized as being involved in anaerobic fermentation and in degradation of cellulose (van Gylswyk 1980). Alligators frequently consume plant-derived organics during feeding. While not yet demonstrated, the initial source of Fusobacteria inoculating alligators may be the vegetation used in nest construction, with the first inoculants being hatchlings. Alligators’ nests are constructed of local vegetation, including *Spartina patens* (Joanen 1969). The high cellulose content of this nest material would suggest
presence of cellulose-degrading microorganisms, which may contain members of Fusobacteria. Unraveling the initial source of members of Fusobacteria in alligators’ microbiome is an unexplored and promising avenue for future discoveries related to the acquisition of a microbiome, as well as to the development of the immune system.

The ability of Fusobacteria to form biofilms in the human mouth (e.g., Munson et al. 2004) suggests that they may be functioning in a similar manner in the gut of alligators. Additionally, Roggenbuck et al. (2014) recently suggested that the presence of this phylum in the two vertebrate taxa, alligators and vultures, both of which may scavenge partially decomposed prey (e.g., Platt et al. 2014), likely reflects a competitive advantage of Fusobacteria in the lower GI tract, and suggests tolerance of toxins produced by pathogens like Clostridia in decomposing food. Fusobacteria are often pathogenic in humans (e.g., Langworth 1977), but for vultures, Fusobacteria are suggested to provide critical functions for the host’s health, immunity, and potentially for the uptake of nutrients through the breakdown of carrion (Roggenbuck et al. 2014). We suggest that Fusobacteria may benefit alligators in a similar way, given both the similarity in diet as well as the numerical abundance of Fusobacteria in their GI tracts compared with vultures. However, until this phylum is studied more fully for the range of vertebrate hosts, its functional role(s) will remain uncertain.

Microbiomes of non-mammalian vertebrates

The greatest amount of research into vertebrates’ microbiomes has focused almost exclusively on mammals, more specifically humans, despite representing just a single vertebrate species. This work continues to provide novel insights into health (Cho and Blaser 2012) and disease (Ley et al. 2005; Turnbaugh et al. 2009; Subramanian et al. 2014), and the intimate association between a vertebrate and its microbial symbionts. Unsurprisingly, the majority of studies of the microbiomes of non-mammal vertebrates relate directly to agriculture, the diet of humans, animal husbandry (particularly of broiler chickens) (e.g., Bjerrum et al. 2006), and aquaculture (e.g., Llewellyn et al. 2014).

In addition to research on the microbiome of the American alligator (Keenan et al. 2013; Keenan 2014a, 2014b) (Fig. 3) there are a limited, but growing, number of studies focusing on other reptiles, e.g., Burmese pythons (Costello et al. 2010), marine and terrestrial iguanas, giant tortoise (Hong et al. 2011; Lankau et al. 2012), and on amphibians, e.g., leopard frogs (Kohl et al. 2013) and various species from Northern California (Kueneman et al. 2014) (Table 2). The insights gained from reptilian, amphibian,
<table>
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<th>Vertebrate host (sample location)</th>
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<tr>
<td>Alligator, <em>Alligator mississippiensis</em> (feces)</td>
<td>Keenan (2014a)</td>
<td>9</td>
<td>69.6</td>
<td>16.7</td>
<td>5</td>
<td>2.7</td>
<td>0</td>
<td>0.08</td>
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<td>0</td>
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<td>Burmese python (fasted, pooled GI tract)</td>
<td>Costello et al. (2010)</td>
<td>3</td>
<td>0.5</td>
<td>61.8</td>
<td>20.6</td>
<td>10.1</td>
<td>0</td>
<td>0.6</td>
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<td>3.9</td>
<td>0.6</td>
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<tr>
<td>Land iguana (feces)</td>
<td>Hong et al. (2011)</td>
<td>16</td>
<td>0.6</td>
<td>63.9</td>
<td>4.2</td>
<td>1.4</td>
<td>0</td>
<td>1.3</td>
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<td>Marine iguana (feces)</td>
<td>Hong et al. (2011)</td>
<td>30</td>
<td>0</td>
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<td>8.2</td>
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<td>0</td>
<td>0.6</td>
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<td>Giant tortoise (feces)</td>
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<td>4</td>
<td>0</td>
<td>81.1</td>
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<td>2</td>
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<td>Green iguana (feces)</td>
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<td>2</td>
<td>0</td>
<td>74</td>
<td>10.1</td>
<td>3.1</td>
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<td>Northern leopard frog tadpole, <em>Lithobates pipiens</em> (intestine)</td>
<td>Kohl et al. (2013)</td>
<td>7</td>
<td>0.01</td>
<td>36.6</td>
<td>2.43</td>
<td>54.9</td>
<td>0.09</td>
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<td>Kohl et al. (2013)</td>
<td>8</td>
<td>0.32</td>
<td>66.1</td>
<td>22.8</td>
<td>10.4</td>
<td>0</td>
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<td>Western toad, <em>Anaxyrus boreas</em> (skin)</td>
<td>Kueneman et al. (2014)</td>
<td>47</td>
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<td>3.7</td>
<td>8.9</td>
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<td>California newt, <em>Taricha torosa</em> (skin)</td>
<td>Kueneman et al. (2014)</td>
<td>16</td>
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<td>3.0</td>
<td>3.7</td>
<td>74.7</td>
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<td>30</td>
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<td>Kueneman et al. (2014)</td>
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<td>Vultures</td>
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<tr>
<td>Black vulture (gut)</td>
<td>Raggenbuck et al. (2014)</td>
<td>26</td>
<td>21.2</td>
<td>67.1</td>
<td>0</td>
<td>11.2</td>
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<td>Black vulture (skin)</td>
<td>Raggenbuck et al. (2014)</td>
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<td>6.1</td>
<td>34</td>
<td>12.1</td>
<td>22.6</td>
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<td>0</td>
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<td>8.9</td>
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<td>Turkey vulture (gut)</td>
<td>Raggenbuck et al. (2014)</td>
<td>23</td>
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<td>60.8</td>
<td>0</td>
<td>5.4</td>
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<td>1.5</td>
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<td>Turkey vulture (skin)</td>
<td>Raggenbuck et al. (2014)</td>
<td>24</td>
<td>3.0</td>
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<td>31.3</td>
<td>0</td>
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and avian species have the potential to transform our view of microbiomes. For example, in alligators and more recently in vultures, Fusobacteria dominated the biota of the microbiome (Keenan et al. 2013; Roggenbuck et al. 2014), hinting that diet might be exerting a large control on the biome’s composition. Both the co-evolved microbial assemblages and the vertebrate host preserve two evolutionary records. One of the goals of research on microbiomes is to utilize the evolutionary record of acquisition of microbial assemblages to decipher the evolutionary relationships among vertebrate hosts (e.g., Ochman et al. 2010). However, the question then stands as to what extent can the composition of microbiomes be used to reconstruct vertebrate hosts’ phylogenetic affiliations? The discovery that microbiome composition of hominids directly correlated with hominid phylogenetic relationships opened the door to using the microbiome as a tool to evaluate the evolution of vertebrates (Ochman et al. 2010). Using the current body of knowledge on composition of the microbiomes of reptiles and amphibians, it is possible to begin to address questions related to membership in microbial assemblages and to hosts’ evolutionary relationships. For example, with the available data it is possible to ask basic questions relating to the similarity or differences in microbiome membership as a function of hosts’ phylogenetic relationships, diet, or environment. These comparisons can help elucidate the presence of shared, ancestral microbial assemblages, as well as identify the acquisition of more recent symbiotic microorganisms.

Data obtained from the American alligator (Keenan et al. 2013) were compared with those available in the published literature for other reptilian and amphibian taxa through May 2014 (Table 2). The resulting dataset incorporated six reptilian and six amphibian taxa. In addition, data recently available from the microbiome of vultures were also included as similarities were noted in the abundance of Fusobacteria (Roggenbuck et al. 2014). Datasets were deemed adequate for comparative purposes if one of the goals of the prior study was to evaluate composition of the microbiome, if bacterial phyla were displayed visually or in tables, and if details of type of sample (e.g., gut or skin) were provided.

Despite the limited number of reptilian and amphibian taxa studied to date, interesting patterns are beginning to emerge. As observed in all other vertebrates, there is a “core” assemblage dominated by representatives of Bacteroidetes, Firmicutes, and Proteobacteria (Fig. 4). Compared with other reptiles

![Unconstrained cluster analysis of bacterial phyla composing microbiomes of reptiles and amphibians, based on unpaired groups and Bray–Curtis dissimilarity. Normalized bacterial phyla are presented above the dendogram, highlighting the species-specific variability in the microbiomes’ composition (see Table 2 for references; figure modified from Keenan [2014b]).](https://academic.oup.com/icb/article-abstract/55/6/972/2363255)
and to vultures, the American alligator’s microbiome is distinct due to an overwhelming dominance of Fusobacteria (Fig. 4). Samples obtained from pythons, iguanas, tortoises, and leopard frogs contained a dominance of Firmicutes, with lesser representation of Bacteroidetes. Microbiomes representative of the bullfrog, treefrog, newt, and western toad have a larger dominance of Proteobacteria. If these results were examined out of context of the source of the sample, it could be suggested that perhaps reptiles and amphibians could be distinguished by the relative dominance of Firmicutes or Proteobacteria (Fig. 5A). However, when the source of the sample is included in the analysis, a very different picture emerges in which the influence of the type of sample (e.g., skin swab or the contents of the GI tract) exerts a large control on how samples begin to cluster (Fig. 5B). This represents a significant obstacle for evaluating the composition of microbiomes in broader contexts, including the relative influence of the host’s diet, environment, and genetics on members of the microbial assemblage. Additionally, due to these differences in types or locations of samples, attempts to use microbiome composition as a window into symbioses maintained over time is extremely challenging. Further sampling of the microbiomes of the skin and/or GI tract from additional reptilian and amphibian taxa may make phylogenetic reconstructions an achievable goal.

**Functional characterization of microbes and microbial assemblages**

The potential for identifying microorganisms involved in health or disease relies on an understanding of their metabolic functioning. Technological advances have made characterizing the “who’s there” relatively straight-forward—it’s the “what are they doing” that remains more difficult to answer. For the GI tract’s microbiome, understanding microbial function is not a trivial issue. New analytical approaches, including metagenomics (e.g., Illumina-based analyses; Qin et al. 2010) and transcriptomics (e.g., RNA-Seq; David et al. 2014), are transforming our view of how microbiomes function in humans. In addition to serving a direct role through their metabolic activity, microorganisms have the potential of directly influencing the host through other interactions. The potential for bi-directional communication between microorganisms and their vertebrate host via the “brain–gut axis” suggests that the microbiome may be significantly influencing the host’s metabolic, physiological, and immune functions (Bercik et al. 2012).

Additionally, as part of the forces involved in establishment of the microbiome and its co-evolution with the host, the host has the ability to sense beneficial or harmful microbes through epithelial cells (Artis 2008). Through the incorporation of these novel analytical techniques in studies of alligator microbiomes, elucidating the functional and metabolic role(s) of specific bacteria, particularly members of Fusobacteria, may be possible.

**Conclusions**

The vertebrate microbiome, both of the skin and the GI tract, is vastly underexplored, particularly for
reptiles and amphibians (Fig. 3). Given the integral role of the microbiome for maintaining hosts’ health, characterizing the composition and function of assemblages may aid with diagnosing health or disease. Additionally, the GI tract can be considered as an ecosystem in itself, worthy of exploring for the discovery of potentially new microbial taxa. Based on the current work on alligators’ microbiome, it is highly likely that a similarly distinct microbial consortium will be observed in other crocodilian species, distinct from other vertebrate taxa.

In alligators and other crocodilian taxa, a highly adapted and effective immune system may be due to a tight co-evolution with their resident microbiome. Likely, it is through intimate interactions with their gut microbiome that alligators have thrived in pathogen-rich environments. Future work evaluating the connection between microbial assemblages in the GI tract, acquisition of the assemblage after hatching, and development of the immune system are likely to provide new insights into this enigmatic group of reptiles.

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References


