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DIGESTIVO DE AVES CON DIFERENTES DIETAS

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“Las bacterias de nuestro intestino son nuestras amigas procariotas”

Amelia Pérez

"El mundo está dividido fundamentalmente en bacterias y el resto"

Richard Dawkins

"Los organismos vivos visibles funcionan sólo gracias a sus bien desarrolladas conexiones con la red de vida bacteriana. Toda la vida está embebida en esta red autoorganizadora que incluye complicadas redes de sistemas sensores y de control que tan sólo empezamos a percibir"

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Capítulo 3

RESUMEN

Uno de los ecosistemas más complejos, diversos y menos explorados es el que se encuentra en el tracto gastrointestinal de los animales, y durante los últimos años se han explorado estos ecosistemas. Los datos generados por diversos trabajos sugieren que la conformación de las comunidades bacterianas está influenciada por diversos factores, siendo dos de los más importantes la dieta y la filogenia del hospedero; por lo que podemos asumir que aquellos animales que tienen dietas similares tendrán comunidades bacterianas similares. La mejor manera de explorar esta posibilidad, es comparar las comunidades bacterianas en aves que tienen la misma dieta (néctar) y que tienen orígenes filogenéticos diferentes (Trochilidae y Emberizidae), y compararla con un ave que tiene dieta y origen filogenético diferente (*Passer domesticus*). Las aves nectarívoras se eligieron debido que el néctar es el alimento más simple y con menos variabilidad en la naturaleza, por lo que son un modelo ideal para probar la relación bacterias intestinales – dieta. Para cubrir este objetivo, obtuvimos DNA de las bacterias que se encuentran en intestino de estas aves, mediante pirosecuenciación del gen ribosomal 16S rRNA. Se utilizaron diversos programas bioinformáticos para el análisis de las secuencias ribosomales como CD-Hit, AmpliconNoise y una base de datos especializada (RDP). Nuestros resultados muestran que las comunidades bacterianas en el intestino de las aves de estudio están formadas por entre 10 y 18 Phyla bacterianos, las comunidades bacterianas en el intestino de las aves nectarívoras están dominadas por Proteobacteria, y por Firmicutes y Actinobacteria en pequeños porcentajes. Mientras que en *P. domesticus,* las comunidades bacterianas están compuestas en su mayoría por de los Phyla Firmicutes y Proteobacteria (en diferentes proporciones a lo largo del intestino). El análisis de secuencias indica que las comunidades bacterianas en el intestino de las aves nectarívoras son más diversas (entre 9621 y 10293 OTUs) que las del Gorrión Doméstico (4436 OTUs). Las comunidades bacterianas en el intestino de *Cynanthus latirostris* y *Diglossa baritula*, presentan mayor similitud entre ellas, que con las encontradas en el intestino de *Passer domesticus*, diferencias que pueden ser explicadas por la naturaleza de la dieta, debido a que la mayoría de las bacterias que se encuentran en el intestino de las aves nectarívoras son capaces de metabolizar azúcares simples, mientras que las bacterias que dominan las comunidades bacterianas del intestino de *P. domesticus* pueden metabolizar carbohidratos complejos (como los que componen las semillas). Nuestros datos indican que la diversidad bacteriana en el intestino de las aves de estudio es superior a lo reportado para otras aves o mamíferos con dietas más complejas.

Palabras clave: comunidades bacterianas intestinales, diversidad bacteriana, pirosecuenciación, 16S rRNA , *Cynanthus latirostris, Diglossa baritula, Passer domesticus*

Abstract

One of the most complexes, diverse and unexplored ecosystems is found in the gastrointestinal tract of animals, in recent years have increased the study of those ecosystems. The data generated by various studies suggest that the formation of bacterial communities is influenced by several factors, two of the most important are diet and host phylogeny; so we can assume that those animals that have similar diets have similar bacterial communities. The best way to explore this possibility is to compare bacterial communities in birds with the same diet (nectar) and have different phylogenetic origins (Trochilidae and Emberizidae), and compare those with a bacterial community of gut to bird that has diet and different phylogenetic origin (*Passer domesticus*). Nectivorous birds were chosen because the nectar is the simplest and least variable food in nature, so they are ideal for testing the gut bacteria relationship model - diet. To solve this question, we obtained DNA of the bacteria inhabit the gut from these birds, and we obtained the bacterial sequences for pyrosequencing 16S rRNA gen. We used different bioinformatics software for analysis of ribosomal sequences: AmpliconNoise, Estimates and a specialized database (RDP). Our results shows that the bacterial communities in the gut of the birds are composed for between 10 and 18 bacterial Phyla, the bacterial communities in the gut of nectar feeding birds were dominated for Proteobacteria and Firmicutes and Actinobacteria in small percentages. While the bacterial communities in *P. domesticus* gut were composed by Firmicutes and Proteobacteria (in different percentages along the gut). Our results indicated that the bacterial communities in the gut of Nectar-feeding birds have a major diversity (between 9621 and 10294 OTUs) than in the House Sparrow (4436 OTUs). The bacterial communities in the gut of *C. latirostris* and *D. baritula* were more similar to each other than those in *P. domesticus*. These differences can be explained by the nature of the diet because the majority of the bacteria found in the gut of nectar-feeding birds are capable to metabolize simple sugars, while the bacteria that dominated the gut of *P. domesticus* can be metabolize complex carbohydrates (that composed the majority of the seed). Our data indicate that bacterial diversity in the gut of birds study is higher than reported for other birds or mammals with more complex diets.

Key words: Intestinal bacterial communities, bacterial diversity, pyrosequencing, 16S rRNA, *Cynanthus latirostris, Diglossa baritula, Passer domesticus*.

Capítulo 1

INTRODUCCIÓN GENERAL

COMUNIDADES BACTERIANAS EN EL TRACTO INTESTINAL DE AVES Y MAMIFEROS

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Resumen:

El Tracto Gastro-Intestinal (TGI) de vertebrados mantiene un flujo constante de agua, nutrientes y energía. Por sus características funcionales es un ecosistema densamente poblado por bacterias, arqueas, virus, protozoarios y parásitos. Las bacterias desempeñan diversas funciones en el ecosistema intestinal, sin embargo, se conoce muy poco acerca de su diversidad taxonómica, genética y funcional. Durante los últimos años, y con el desarrollo de diversas técnicas moleculares, se ha comenzado a explorar la dinámica de las comunidades bacterianas en el tracto gastrointestinal (TGI) en diversas especies de aves y mamíferos. La colonización bacteriana comienza antes del nacimiento, el tipo de bacterias que coloniza el intestino no sólo depende de las bacterias que habitan el TGI de los padres, sino de las características genéticas de la cría. Una vez que las bacterias se han establecido en el intestino, comienza un rápido recambio de especies hasta que el ecosistema se vuelve estable. El TGI puede dividirse en diversas zonas funcionales, cada una de las cuales, tiene una diversidad y composición bacteriana diferente. Actualmente existen estudios que sugieren que tanto la dieta como la filogenia son los factores que influencian la composición de las comunidades bacterianas. Las aves nectarívoras son un excelente modelo para tratar de responder a esta incógnita, ya que tienen una dieta relativamente simple (básicamente una solución diluida de azúcares y pobre en minerales y proteínas), además

la nectarivoría está presente en diversas familias de aves. La ecología de las comunidades bacterianas en aves y mamíferos silvestres debe incluir preguntas sobre los factores que influyen en la conformación de las comunidades bacterianas, la descripción de las comunidades bacterianas a lo largo de todo el intestino, y la descripción del papel funcional que tiene cada bacteria en el ecosistema intestinal.

Palabras clave: comunidades bacterianas, ecosistema intestinal, dieta, filogenia, relación dieta-bacterias

Abstract:

The Gastro Intestinal tract (GIT) maintains a constant flow of water, nutrients and energy. The functional role of the GIT, allows it to be densely populated by bacteria, protozoa, Archaea, viruses and parasites. Bacteria play various roles in the intestinal ecosystem; however, little is known about their taxonomic diversity, genetic and functional diversity. In recent years, with the development of various molecular techniques, the dynamics of bacterial communities in the GIT of various birds and mammals have started to be studied. Bacterial colonization of the GIT begins before birth. The type of bacteria that colonize the gut not only depends on the bacteria that inhabit the GIT of the parents, but also on the genetic characteristics of the individual. Once the bacteria become established in the gut, a rapid replacement occurs until the ecosystem becomes stable. The GIT can be divided into several functional zones, each of which has a different bacterial composition and diversity. Currently there are different studies suggesting that both the diet and the phylogeny are the most important factors influencing the composition of bacterial communities in the GIT. The nectarivorous birds are excellent model to try to answer determine the role these factors play in the conformation of bacterial communities. The nectarivorous birds eat the most simple food on earth (a dilute solution of carbohydrates and poor in proteins and minerals), moreover, the nectar feeding birds have representatives in various phylogenetic families. The bacterial ecology in the gut of birds and mammals not only should include questions about the factors that influence the composition of bacterial communities, include as well some description of the bacterial communities along the gut, and the functional description of the role that each bacterial species plays in the intestinal ecosystem.

Key words: Bacterial communities, 16S rRNA, intestinal ecosystem, diet, phylogeny, bacteria-diet relationship

Introducción

Uno de los ecosistemas más complejos es el existente en el tracto gastrointestinal (TGI) de vertebrados, el cual es habitado por bacterias, hongos, protozoarios, virus y arqueas en altas densidades (cerca de 10^{14} microorganismos; Stevens y Hume, 1998; Mackie *et al.,* 1999; Gill *et al.,* 2006; Ley *et al.,* 2006; Rajilić-Stojanović *et al.,* 2007). El conjunto de todos estos microorganismos recibe el nombre de microbiota (Zoetendal *et al.,* 2001; Dethlefsen *et al.,* 2006; Ley *et al.,* 2008). Durante millones de años, se ha desarrollado una compleja y dinámica relación entre el hospedero y los microorganismo alojados en su intestino, en donde ambas partes son beneficiadas (Blaser, 2006; Tellez *et al.,* 2006; Dethlefsen *et al.,* 2007). Se cree que la evolución ha seleccionado aquellas poblaciones microbianas que mantienen o incrementan la adecuación (*Fitness*) del hospedero, y de los demás miembros del ecosistema intestinal como un todo (Blaser, 2006; Egert *et al.,* 2006; Zilber-Rosenberg y Rosenberg, 2008).

Algunos autores han sugerido que la microbiota intestinal debería ser considerada como un órgano extra, debido a que las bacterias intestinales tienen diversas funciones que complementan las funciones del aparato digestivo del hospedero (Bâckhed *et al.,* 2004; Egert *et al.,* 2006; Zilber-Rosenberg y Rosenberg, 2008). Entre estas funciones se encuentran: a) contribución a la digestión enzimática (Randall *et al.,* 2002), b) síntesis de vitaminas (Canny y McCormick, 2008), c) almacenamiento de grasas (Bäckhed *et al.,* 2004; Bäckhed *et al.,* 2006; Zhang *et al.,* 2009), d) eliminación de toxinas y substancias dañinas como taninos y alcaloides (Klasing, 1999; Zoetendal *et al.,* 2006; Dethlefsen *et al.,* 2007), e) metabolismo de diversos carbohidratos (Xu *et al.,* 2003), f) transporte y absorción de agua y electrolitos (Laverty y Skaudhauge, 1999), y g) degradación, absorción y reciclaje de compuestos nitrogenados (Klasing, 1999; DeGolier *et al.,* 1999). Todos estos procesos son importantes nutricionalmente para el hospedero, especialmente el balance de nitrógeno y la síntesis de proteínas en aquellas especies que se alimentan de dietas pobres en proteína o deficientes en ciertas vitaminas (DeGolier *et al.,* 1999; Ohkuma *et al.,* 1999; Press *et al.,* 2003), y finalmente h) del desarrollo del sistema inmune (Walker, 2000).

A pesar de la importancia que las bacterias intestinales tienen para la sobrevivencia de los animales, se conoce muy poco acerca de las relaciones existentes entre las bacterias y su hospedero. En general sólo

conocemos un poco de su diversidad taxonómica y genética, pero carecemos de la información básica sobre el papel funcional que las bacterias tienen en el ecosistema intestinal (Breitbart *et al.,* 2003; Zoetendal *et al.,* 2006). El presente trabajo es una revisión de algunos aspectos del estudio de las comunidades bacterianas en el tracto gastrointestinal de aves y mamíferos, describimos los factores que determinan la composición de las comunidades bacterianas, y finalizamos con una breve descripción de lo que se ha reportado sobre la composición de las comunidades bacterianas en el intestino de aves y mamíferos con diferentes dietas.

Estudio de las comunidades bacterianas intestinales de mamíferos y aves

A pesar de la importancia que las bacterias intestinales tienen en la adecuación del hospedero, sólo conocemos algunos aspectos de su genética, ecología y su papel fisiológico (Breitbart *et al.,* 2003; Zoetendal *et al.,* 2006). Este desconocimiento de la diversidad bacteriana y sus funciones en el TGI se debe principalmente a nuestra incapacidad para cultivar estas bacterias en condiciones de laboratorio. Se ha calculado que menos del 1% de las bacterias pueden crecer en medios de cultivo, debido a que se carece de los sustratos y condiciones anaeróbicas óptimas (Breitbart *et al.,* 2003; Alexander *et al.,* 2006; Streit y Schmitz, 2009). Durante los últimos años el desarrollo de diversas técnicas moleculares, tales, como la secuenciación de Sanger, RISA, la secuenciación masiva del gen 16S rRNA, el uso de DGGE, TRFLP, microarreglos de DNA, FISH, qPCR, y aplicaciones como la metagenómica, metabolómica, metaproteómica y metatranscriptomica (Alexander *et al.,* 2006; Zoetendal *et al.,* 2008; Benskin *et al.,* 2010; Serikov *et al.,* 2010), han permitido la exploración de la diversidad taxonómica y funcional de diferentes ecosistemas bacterianos.

Dos de las técnicas más usadas en el estudio de las comunidades bacterianas son: el Análisis del Espacio Inter Ribosomal (RISA por sus siglas en inglés) y la amplificación del gen 16S rRNA. El análisis RISA mide el espacio existente entre los operones del 16S y el 23S rRNA, espacio altamente variable entre especies bacterianas (Nagpal *et al.,* 1998; White *et al.,* 2010). El uso de esta técnica permite la comparación de comunidades bacterianas de diferentes muestras a un bajo costo. Sin embargo, los resultados obtenidos cuando se utiliza RISA son difíciles de interpretar, debido a que existe variabilidad en el tamaño de los espacios ribosomales dentro de una misma especie bacteriana (Gürtler y Stanisich, 1996; Ruiz-Rodríguez *et al.,* 2009;

Serikov *et al.,* 2010). Adicionalmente, esta técnica no permite la identificación taxonómica de las bacterias que componen la muestra (Gürtler y Stanisich, 1996; Ruiz-Rodríguez *et al.,* 2009; Serikov *et al.,* 2010).

Por otro lado, la amplificación y secuenciación masiva del gen ribosomal 16S rRNA tiene ventajas sobre la técnica de RISA, y es de bajo costo en comparación con el número de secuencias que permite obtener (entre 200 000 y 1 000 000). Existen dos tecnologías para la secuenciación masiva del gen 16S rRNA: la pirosecuenciación (454 Life Sciences) y la secuenciación reversible (Solexa / Illumina). Ambas tecnologías, permiten la obtención de un gran número de secuencias, de tamaño variable (de entre 100 y 500 pb por muestra; Liu *et al.,* 2007; Cárdenas y Tiedje, 2008; Serikov *et al.,* 2010), en un corto tiempo y permite el análisis de muestras múltiples en una corrida de secuenciación usando primers marcados con un código de barras (Armougom y Roult, 2008; Hamady *et al.,* 2008). Los datos obtenidos permiten la comparación de comunidades bacterianas de diversas muestras utilizando una amplia variedad de programas bioinformáticos, entre los que destacan UniFrac (Lozupone *et al.,* 2005; Lozupone *et al.,* 2007; Anderson *et al.,* 2008), Dotur (Schloss y Handelsman, 2005; Samsudin *et al.,* 2011), LIBSHUFF (Wang *et al.,* 2007; Sundset *et al.,* 2007), MG- Rast (Meyer *et al.,* 2008) y AmpliconNoise (Quince *et al*., 2011) y bases de datos especializadas como Greengenes (http://greengenes.lbl.gov/cgi-bin/nph-index.cgi), RDP (http://pyro.cme.msu.edu/), KEGG (Kyoto Enclyclopedia of Genes and Genomes, http://www.genome.jp/kegg/) y NCBI (http://www.ncbi.nlm.nih.gov/), entre otras.

La pirosecuenciación ha recibido críticas debido a que puede subestimar y/o sobreestimar la diversidad bacteriana presente en la muestra. Esto se da por errores de secuenciación de regiones homopoliméricas, la formación de quimeras y la amplificación preferencial de algunos grupos bacterianos (Palmer *et al.,* 2007; Wei *et al.,* 2007; Armougom y Roult, 2008; Oh *et al.,* 2010). El tamaño de las secuencias obtenidas también ha sido motivo de controversia, ya que algunos autores argumentan que secuencias pequeñas (100 – 500 pb) no pueden ser identificadas correctamente (Liu *et al.,* 2007; Anderson *et al.,* 2008; Cardenas y Tiedje, 2008; Serikov *et al.,* 2010). Sin embargo, el trabajo realizado por Cárdenas y Tiedje (2008) demuestra que las secuencias bacterianas analizadas en la RDP (Ribosomal Database Project), necesitan una longitud mínima de 200 pb para ser identificadas correctamente a nivel de familia, y de 400 pb para género (Cárdenas y Tiedje, 2008). La identificación a nivel de especie es complicada, debido a que sólo se ha secuenciado una pequeña parte de la gran diversidad bacteriana existente, además, que hasta el momento no existen límites claros en las

diferencias genéticas entre los miembros del mismo género (% de diferencia genética en uno o varios genes; Konstantinidis y Tiedje, 2005; Cohan 2006; 2007; Stackebrandt y Ebers; 2006), y a que no existen los recursos bioinformáticos adecuados (Nagpal *et al.,* 1998; Ronaghi *et al.,* 2000; 2001; Heilig *et al.,* 2002),

La secuenciación masiva del 16S rDNA, es la técnica más utilizada y ha permitido la descripción de ecosistemas bacterianos de diversos ambientes. Uno de los ambientes que se han explorado es el tracto gastrointestinal de los vertebrados. En la última década se inició la exploración de ecosistemas intestinales de algunos mamíferos de los Órdenes Artiodactyla (Hartel *et al.,* 2003; Nelson *et al.,* 2003; Sundset *et al.,* 2004; Saengkerdsub *et al.,* 2006; Wood *et al.,* 2006), Carnivora (Wei *et al.,* 2007; Glad *et al.,* 2010); Primates (Breitbart *et al.,* 2003; Eckburg *et al.,* 2005; Dethlefsen *et al.,* 2006; Frey *et al.,* 2006; McKenna *et al.,* 2008), Rodentia (Akada *et al.,* 2003; Sarma-Rupavtarm *et al.,* 2004; Bäckhed *et al.,* 2005; Alexander *et al.,* 2006; Coolon *et al.,* 2010); además de 60 especies de mamíferos de diversos órdenes (Ley *et al.,* 2008). También se describieron comunidades bacterianas en el intestino de aves de corral, del Orden Galliformes (Zhu *et al.,* 2002; Lu *et al.,* 2003; Gong *et al.,* 2007), y algunas aves silvestres de los Órdenes Charadriformes (White *et al.,* 2010), Passeriformes (Lucas *et al.,* 2005) y Sphenisciformes (Banks *et al.,* 2009).

Estos estudios muestran que las comunidades bacterianas del tracto intestinal de mamíferos y aves están compuestas por miembros de al menos 20 Phyla bacterianos, además se ha reportado la presencia de Archaea (Ley *et al.,* 2008; McKenna *et al.,* 2008; Tabla 1.1), aunque uno de los estudios más completos hechos en el ser humano, indica que las comunidades bacterianas pueden estar compuestos por 70 Phyla (Turnbaug *et al*., 2007). No obstante la diversidad de Phyla bacterianos presentes en los ecosistemas intestinales, las comunidades bacterianas de este ambiente están dominadas por Firmicutes y Bacteroidetes (Bäckhed *et al.,* 2005; Rajilić-Stojanović *et al.,* 2007; Zilber-Rosenberg y Rosenberg, 2008). Sin embargo, la composición de las comunidades bacterianas intestinales, a nivel de género y especies puede ser variable (Eckburg *et al.,* 2005; Gill *et al.,* 2006). Esto sugiere, que la presión selectiva favorece la permanencia de ciertos Phyla, mientras que la redundancia funcional permite un aumento en la diversidad bacteriana, sin comprometer el mantenimiento de las funciones del ecosistema intestinal (Zoetendal *et al.,* 2006; Serikov *et al.,* 2010). También es probable que exista un alto grado de especialidad funcional de ciertos grupos bacterianos a las condiciones presentes en los tractos digestivos (Turroni *et al.,* 2008). De este modo, el número y tipo de especies bacterianas presentes en

un ecosistema intestinal, así como su tamaño poblacional, podría ser diferente para cada ambiente que existe en el intestino, sin que la funcionalidad del ecosistema completo se vea afectada (Sundset *et al.,* 2004; 2007). Mucho se ha especulado acerca de cuáles son los factores que determinan o influyen en la conformación de las comunidades bacterianas en el tracto gastrointestinal de los vertebrados, a continuación se hará una breve revisión sobre ese tema.

Tabla 1.1 Phyla bacterianos presente en el intestino de aves y mamíferos

Phyla	Referencia
Acidobacteria	McKenna *et al.,* 2008
Actinobacteria	Leser *et al.,* 2000; Daly *et al.,* 2001; Scupham *et al.,* 2008; Becker *et al*., 2014
Bacteroidetes	Daly *et al., 2*001; Tajima *et al.,* 2001; An *et al.,* 2005; Sundset *et al., 2*007
Chlamydiae	Carroll *et al.,* 2009
Chloroflexi	Carroll *et al.,* 2009, Davenport *et al.,* 2014
Cyanobacteria	Ley *et al.,*2008; McKenna *et al.,* 2008
Deferribacteres	Preest et al., 2003; McKenna *et al.,* 2008; Scupham *et al.,* 2008
Deinococcus-Thermus	Carroll *et al.,* 2009
Elusimicrobia	Carey *et al*., 2012
Firmicutes	Daly *et al.,* 2001; Tajima *et al.,* 2001; An *et al.* 2005
Fusobacteria	Lu *et al.,* 2003; Becker *et al*., 2014
Gemmatimonadetes	Davenport *et al.,* 2014
Lentisphaerae	Leser *et al.,* 2000; Frey *et al.,* 2006
Melainabacteria	Rienzi *et al*., 2014
Planctomycetes	Leser *et al.,* 2000; Frey *et al.,* 2006, Bolnick *et al.,* 2013
Proteobacteria	Daly *et al.,* 2001; Tajima *et al., 2*001; An *et al.,* 2005; Sundset *et al.,* 2007
SR1	An *et al.,* 2005; Sundset *et al.,* 2007; Kong *et al.,* 2010; Samsudin *et al.,* 2011
Spirochaetes	Leser *et al.,* 2000; Tajima *et al.,* 2001; Daly *et al.,* 2001; Godoy-Vitorino *et al.,* 2008
TM7	Eckburg *et al.,*2005; Bik *et al.,* 2006; Godoy-Vitorino *et al.,* 2008
Verrucomicrobia	Daly *et al.,* 2001; Eckburg *et al.,* 2005; Godoy-Vitorino *et al.,* 2008, Davenport *et al*., 2014.

Factores que afectan la composición de las comunidades bacterianas intestinales

Los resultados de diversos autores sugieren que la composición de las comunidades bacterianas es afectada por diferentes factores como: la colonización bacteriana, cambios microambientales del ecosistema intestinal (Stevens y Hume, 1998; Sonnenburg *et al.,* 2005; Alexander *et al.,* 2006), las características genéticas del hospedero (Zoetendal *et al.,* 2001; Mai y Morris, 2004; Zoetendal *et al.,* 2006; Benson *et al., 2*010), la porción del tracto intestinal analizada (Michalet-Doreau *et al.,* 2002; Hayashi *et al.,* 2005; McKenna *et al.,* 2008), la

presencia de infecciones o enfermedades (McKenna *et al.,* 2008), la ingesta de antibióticos (Panda *et al.,* 2014), la edad del hospedero (Lu *et al.,* 2003; Zoetendal *et al.,* 2006), sexo (Bolnick *et al.,* 2013), las relaciones filogenéticas de los hospederos (Maul *et al.,* 2005; Ley *et al.,* 2008; Glad *et al.,* 2010), los ciclos circadianos (Carey *et al.,* 2012), la dieta (Tajima *et al.,* 2001; Lu *et al.,* 2003; Sundset *et al.,* 2004; Wei *et al.,* 2007; McKenna *et al.,* 2008). A continuación se describen brevemente cada uno de estos factores.

Establecimiento de las comunidades bacterianas

El ecosistema intestinal presenta una gran diversidad de condiciones fisicoquímicas (pH, secreciones glandulares, y flujos y tipos de nutrientes presentes en el ambiente a lo largo del intestino; Stevens y Hume, 1998; Hooper *et al.,* 1999; Sonnenburg *et al., 2*005; Brzuskewicz *et al.,* 2006) y estructurales (conformación anatómica del intestino), por lo que la disponibilidad de nichos dentro del ecosistema intestinal es enorme, y las probabilidades de colonización bacteriana aumentan (Marteau *et al.,* 2001; Eckburg *et al.,* 2005; Turnbaugh *et al.,* 2006). Actualmente se desconocen los mecanismos por los cuales las bacterias colonizan el TGI. Hasta hace poco, se consideraba que el intestino era un ambiente estéril durante el desarrollo embrionario y hasta antes del nacimiento (Stevens and Hume, 1998) y que la colonización bacteriana comenzaba poco después del nacimiento (Eckburg *et al*. 2005; Zoetendal *et al*., 2008; Sommer y Bäckhed, 2013). Sin embargo, estudios recientes muestran que el intestino es colonizado aún antes del nacimiento (Ardissone *et al*., 2014; Cacho y Neu 2014; Moles *et al*., 2014); estudios muestran que en el tejido placentario y el líquido amniótico presentan bacterias en un número importante (Ardissone *et al.,* 2014). Existen varios mecanismos de colonización amniótica, entre los cuales se encuentra la ascensión y translocación de microbiota vaginal (Goldenberg *et al*., 2008) y por vía sanguínea (Goepfert *et al.,* 2004). Se sabe que los bebés humanos toman pequeñas cantidades de líquido amniótico, proceso que da inicio a la colonización bacteriana. Los resultados de Ardissone y colaboradores (2014), corroboran estos resultados, ya que al examinar el meconio de infantes de 33 semanas de gestación encontraron que el 61.1% de las bacterias presentes se encontraban en el líquido amniótico. La composición de las comunidades bacterianas en el meconio cambia rápidamente durante la primera semana posterior al nacimiento (Moles *et al.,* 2014)

En aves, la colonización del TGI, comienza durante el periodo de la incubación, ya que algunas bacterias pueden atravesar el cascarón de los huevos de gallinas (*Gallus gallus domesticus*; De Reu *et al.,* 2006). Posteriormente, una nueva fase de la colonización bacteriana ocurre cuando el pollo eclosiona y entra en contacto con el nido, el cual es considerado un reservorio de las bacterias que habitan en la cloaca de los padres (Mills *et al.,* 1999; Maul *et al.,* 2005). La siguiente fase, ocurre cuando los padres alimentan a los pollos, ya que el alimento regurgitado es una mezcla de alimento, saliva y bacterias (Salanitro *et al.,* 1978; Mills *et al.,* 1999; Godoy-Vitorino *et al.,* 2010). De este modo la colonización bacteriana del tracto gastrointestinal de las aves parece tener un fuerte componente vertical, ya que las comunidades bacterianas pasan de padres a hijos a través de la crianza en el nido. Finalmente, algunas bacterias son adquiridas por coprofagia (Stevens y Hume, 1998; Mills *et al.,* 1999) y otras a través del ambiente (Stewart y Rambo, 2000; Lovanh *et al.,* 2007; Banks *et al.,* 2009).

En el caso de los mamíferos, la colonización bacteriana en las etapas posteriores al nacimiento es tanto vertical como horizontal. El componente vertical está relacionado con la trasmisión de bacterias de madre a hijo cuando la cría atraviesa el canal de parto durante el nacimiento (Newburg, 2000; Blaser, 2006; Palmer *et al.,* 2007; Zilber-Rosenberg y Rosenberg, 2008), durante la lactancia (Palmer *et al.,* 2007; Cabrera-Rubio *et al.,* 2012) y a través de la saliva de la madre (Newburg, 2000; Dethlefsen *et al.,* 2007; Palmer *et al.,* 2007). El componente horizontal se da con el contacto que la cría tiene con otros individuos (Newburg, 2000; Zilber-Rosenberg y Rosenberg, 2008), además de que múltiples bacterias son adquiridas directamente del ambiente (Zilber-Rosenberg y Rosenberg, 2008).

La adquisición de bacterias intestinales se mantiene a lo largo de toda la vida de los organismos. En este proceso tanto los padres, abuelos, niñeras, hermanos, parejas reproductivas o cualquier otro organismo que esté en contacto con un individuo, puede transferir microorganismos que habiten en el TGI, influyendo en la composición de sus comunidades bacterianas (Dethlefsen *et al.,* 2007; Zilber-Rosenberg y Rosenberg, 2008; Oh *et al.,* 2010). Tanto en aves como en mamíferos, las comunidades bacterianas son similares en individuos provenientes de la misma familia (Zoetendal *et al.,* 2001; Lucas *et al.,* 2005; Dethlefsen *et al.,* 2007). Sin embargo, es probable, que más que la convivencia de los miembros de una familia, la colonización bacteriana

está influenciada por las características genéticas del hospedero (Zoetendal *et al.,* 2001; Lu *et al.,* 2003; Ley *et al.,* 2006; Scupham *et al.,* 2008).

Aparentemente las bacterias pioneras, pueden modular la expresión de genes del hospedero para crear ambientes más favorables y prevenir el crecimiento de otras bacterias en el ecosistema (O'Hara y Shanahan, 2006). Una vez que las bacterias se han establecido en el intestino, los sustratos disponibles en el ecosistema, pueden influir en la composición de las comunidades bacterianas durante los primeros meses de vida de la cría (Godoy-Vitorino *et al.,* 2010; Sommer y Bäckhed, 2013). Por ejemplo, bebés humanos alimentados con leche materna tienen comunidades bacterianas dominadas por *Enterobacteria y Bifidobacteria*; mientras que los bebés alimentados con fórmulas lácteas, tienen comunidades dominadas por *Bifidobacteria, Clostridia*, *Bacteroides*, y *Streptococci* (Newburg, 2000; O'Hara y Shanahan, 2006). La composición de las comunidades bacterianas pueden cambiar si existe alguna infección durante el período de colonización (Newburg, 2000; O'Hara y Shanahan, 2006; Palmer *et al.,* 2007) y con la ingesta de antibióticos (Panda *et al.,* 2014).

Algunos estudios muestran que la competencia por los nichos dentro del intestino es intensa y actúa junto con otras fuerzas selectivas que promueven el mutualismo entre la comunidad bacteriana y su hospedero, por lo que las bacterias al competir, tienden a cooperar de manera simultánea (Dethlefsen *et al.,* 2007). Por ejemplo, la competencia entre patógenos puede disminuir la colonización de otros patógenos, mediante la producción de sideróforas (moléculas transportadoras de hierro). Estas moléculas provocan una reducción en la virulencia de las bacterias patógenas que se alimentan de ellas, lo que beneficia al hospedero (Dethlefsen *et al.,* 2007). Otra forma en que algunos patógenos inhiben la presencia de otros patógenos, es la activación de sensores que activan la secreción de citoquinas y quimosinas, sustancias que alertan y activan el sistema inmune del hospedero (O´Hara y Shanahan, 2006).

Una vez que las primeras bacterias arriban al intestino, comienza un rápido recambio de especies (Davila *et al*., 2003; Moles *et al*., 2014). Un análisis del proceso de colonización y sucesión de las comunidades bacterianas en recién nacidos humanos, encontró cambios importantes a lo largo del primer año de vida (Zoetendal *et al.,* 2001; Sommer y Bäckhed, 2013; Cacho y Neu 2014). Durante las etapas iniciales, la microbiota encontrada fue relativamente simple, con cambios caóticos en su composición en los siguientes meses de vida, hasta que finalmente se vuelve una comunidad estable, compleja y similar a la de la madre al

final del estudio (Zoetendal *et al.,* 2001; Palmer *et al.,* 2007). La formación de comunidades bacterianas estables en el TGI depende de la adquisición selectiva de bacterias ambientales (Newburg, 2000). La estabilidad de la comunidad ha sido estudiada en muestras tomadas durante semanas, meses e incluso años (Franks *et al.,* 1998; Zoetendal *et al.,* 1998; Zoetendal *et al.,* 2001). Aún no existe un acuerdo con respecto al tipo de muestras y el período de muestreo óptimo, que represente de manera adecuada la variabilidad en la composición de las comunidades intestinales a corto, mediano o largo plazo (Zoetendal *et al.,* 2001; Dethlefsen *et al.,* 2006). A pesar de que algunos autores piensan que la transmisión de las bacterias que colonizan el TGI puede ser incidental (Palmer *et al.,* 2007), la evidencia sugiere que el establecimiento de nuevas bacterias adquiridas en el ambiente externo al TGI puede estar influenciado a las especies bacterianas que colonizaron primero, y controlado por las características genéticas del hospedero (Walker, 2000; Guan *et al.,* 2008; Li *et al.,* 2008; Sommer y Bäckhed, 2013).

Características genéticas del hospedero:

Algunos autores sugieren que las características genéticas de hospedero pueden determinar el tipo de bacterias que se establezcan dentro del intestino (Walker, 2000; Guan *et al.,* 2008; Li *et al.,* 2008). La de una nueva colonia de bacterias está determinada por los receptores que se encuentran en las membranas celulares que recubren el epitelio intestinal, en un mecanismo similar al de llave - cerradura (Newburg, 2000). Esto significa que para que una bacteria pueda ocupar un nicho, los receptores específicos glicoconjugados de la membrana de las bacterias deben unirse a sus contrapartes que se encuentran en los vellos y membranas intestinales del hospedero (Hooper *et al.,* 1999; Walker, 2000; Akada *et al.,* 2003; Becker y Lowe, 2003; Dethlefsen *et al.,* 2006, Serikov *et al.,* 2010). Benson y colaboradores (2010) examinaron la relación existente entre la genética del hospedero y la composición de las comunidades bacterianas en el intestino de ratones (*Mus musculus*) que pertenecían a diferentes líneas genéticas. Estos autores, midieron la variación alélica de caracteres cuantitativos (Quantitative Trait Locus) para probar si taxones específicos de bacterias con herencia paralela de genes que se encuentran localizados en el mismo cromosoma (co-segregación), se ligan con marcadores genómicos específicos del hospedero. Sus resultados indican que las interacciones bacteria -

hospedero, tienen tres mecanismos de acción dependiendo de los loci presentes en las bacterias y el hospedero. El primer mecanismo permite la colonización de bacterias específicas, el segundo el establecimiento de grupos de bacterias filogenéticamente relacionadas, mientras que el tercero tiene efectos pleiotropicos sobre grupos bacterianos lejanamente relacionados. Por lo tanto, la presencia o ausencia de estos loci determinan la diversidad del microbioma intestinal (Benson *et al.,* 2010).

Otro mecanismo genético involucrado en el control de la composición de las comunidades bacterianas es el Complejo Mayor de Histocompatibilidad (MHC, por sus siglas en inglés). Dicho complejo está conformado por un conjunto de genes polimórficos cuyos productos son expresados en la superficie de las células del sistema inmune (López-Martínez *et al.,* 2005). El polimorfismo en estos genes evoluciona constantemente permitiendo una selección sobre comensales y bacterias patógenas (Detlefsen *et al.,* 2006). Aparentemente las bacterias que llegan primero al ecosistema intestinal pueden modular la expresión de ciertos genes en el hospedero para crear un ambiente favorable para ellas, previniendo el crecimiento de otras bacterias que arriban después al ecosistema gastrointestinal (Zoetendal *et al.,* 2001; Alexander *et al.,* 2006; Dethlefsen *et al.,* 2006; O´Hara y Shanahan, 2006).

Distribución de las bacterias en el Tracto Gastrointestinal:

Aunque existen pocos trabajos que hayan descrito la composición de las comunidades bacterianas a lo largo del TGI de un organismo (Stearn *et al.,* 2011), existen muchos otros que han evaluado la diversidad bacteriana en las diversas regiones del TGI en humano (Paster *et al.,* 2001; Eckburg *et al.,* 2005; Hayashi *et al.,* 2005; Bik *et al.,* 2006; Dethlefsen *et al.,* 2006; Egert *et al.,* 2006; Rajilić-Stojanović 2007; Anderson *et al.,* 2008; Canny y MacCormick, 2008; Contreras *et al.,* 2010; Zhang *et al.,* 2010; Stearn *et al.,* 2011; Belda-Ferre *et al.,* 2012), y en pollos de granja (Gong *et al.,* 2002; Zhu *et al.,* 2002; Gong *et al.,* 2007). Aun cuando los datos son insuficientes para establecer un patrón claro, se pueden observar cambios en el número de especies bacterianas en cada región del TGI (Tabla 1.2), con una diversidad considerable en los ciegos, lo cual hace sentido si consideramos que la función de estas estructuras es la digestión fermentativa (Braun y Campbell, 1989; Zhu *et al.,* 2002; Gong *et al.,* 2007).

La gran mayoría de los estudios que describen las comunidades bacterianas se enfocan en la región correspondiente al intestino (específicamente: excretas). El intestino está dividido en tres zonas funcionales. En la primera ocurre preponderantemente la digestión, mientras que en la segunda se absorben nutrientes, y en la tercera se recupera agua y se preparan los desechos (Stevens y Hume, 1998; Klassing, 1999; Evans, 2004). Cada una de esas zonas presenta diferentes gradientes microambientales (pH, flujo de oxígeno y disponibilidad de nutrientes; Hill, 1981; Sarma-Rupavtarm *et al.,* 2004; Serikov y Finlay, 2006). Por ejemplo, la primera sección del intestino tiende a tener un ambiente más ácido que las otras secciones (pH 5-6 en lugar de un pH de 7). De acuerdo a las características fisicoquímicas y morfológicas que el TGI presenta en sus diferentes regiones funcionales, el tamaño poblacional y la composición y distribución de las comunidades bacterianas deberían ser diferentes en cada porción del tracto gastrointestinal (Zoetendal *et al.,* 2001; Mai y Morris, 2004; Sarma-Rupavtarm *et al.,* 2004).

Tener información confiable sobre los cambios en composición de las comunidades bacterianas a lo largo del intestino puede ayudarnos a responder muchas preguntas, sobre todo de tipo funcional, al relacionar el área funcional del TGI con las bacterias presentes. De este modo llevar a cabo estudios comparativos sobre la estructura de comunidades bacterianas a lo largo del TGI es crucial para poder comprender cuál es el papel de las bacterias en la digestión, el papel de las bacterias en la elección de la dieta por parte de su hospedero, y la diversidad real de las bacterias en este complejo ecosistema. Adicionalmente trabajos con un mayor grado de detalle nos permitirán saber qué tan similares son las comunidades bacterianas entre individuos de la misma especie y si los hospederos con dietas similares tienen comunidades bacterianas similares. Actualmente no tenemos suficiente información para responder a esta y otras preguntas. La razón de esto, es que existen marcadas diferencias en los resultados reportados en la literatura, en el tipo de muestra usada en los análisis (lavado intestinal, raspado, biopsia, heces; Sarva-Rupavtarm *et al.,* 2004; Zoetendal *et al.,* 2006; Xu *et al.,* 2007; Stearns *et al.,* 2011), el método de extracción de DNA usado (kits vs. métodos tradicionales; McOrist *et al.,* 2002; Darling y Blum, 2007; Morgan *et al.,* 2010), las condiciones de PCR (Contreras *et al.,* 2010; De Filippo *et al.,* 2010), porcentaje de similitud para asignar identidad taxonómica de las secuencias para determinar cada OTU bacteriana (Edwards *et al.,* 2004; Lozupone *et al.,* 2008; Zoetendal *et al.,* 2008), y los programas bioinformáticos para hacer el análisis de datos: UniFrac (Lozupone *et al.,* 2005; Lozupone *et al.,* 2007;

Anderson *et al.,* 2008), Dotur (Schloss y Handelsman, 2005; Samsudin *et al.,* 2011), LIBSHUFF (Sundset *et al.,* 2007; Wang *et al.,* 2007), AmpliconNoise (Quince *et al*., 2011); o las bases de datos usadas para asignar función o identidad taxonómica a las secuencias: RDP (An *et al*., 2005; Cole *et al*., 2009), NCBI (http://www.ncbi.nlm.nih.gov/) Greengenes (http://greengenes.lbl.gov/cgi-bin/nph-bel3_interface.cg; Gong *et al*., 2007; Wei *et al.,* 2007), MG- Rast (Meyer *et al*., 2008))

Quizá una de las mayores limitantes en la descripción de las comunidades bacterianas es el tipo de muestra usada para el análisis. En el ambiente intestinal, las bacterias pueden establecerse en diferentes "nichos" intestinales, y dependiendo las características del área física donde se encuentren, pueden hacerlo como: a) comunidades biopelículas ("biofilms"; Cacho y Neu, 2014), b) en la superficie de la pared intestinal íntimamente ligada al tejido epitelial, c) la capa de mucosidad que cubre los vellosidades, d) libres en el lumen intestinal, e) habitando en las vellosidades, plicas y criptas intestinales, y f) pegadas a partículas del alimento (Miron *et al.,* 2001; Rabiu y Gibson, 2002; Akada *et al.,* 2003; Manco *et al.,* 2010). Debido a la inaccesibilidad de los órganos que componen el tracto gastrointestinal, y basándose en el supuesto que todas las células bacterianas que habitan en las paredes intestinales "caen" al torrente intestinal junto con las heces, y abandonan el intestino a través de la cloaca (en aves) o el ano (mamíferos), la mayor parte de los estudios se han llevado a cabo utilizando heces fecales. Esto también se ha justificado debido a que las bacterias intestinales componen entre el 40 y 55 % de la materia fecal (Tellez *et al.,* 2006). Adicionalmente al análisis de heces fecales, se ha cuantificado la diversidad de las comunidades intestinales en el TGI utilizando lavados cloacales o lavados intestinales (Sarva-Rupavtarm *et al.,* 2004; Zoetendal *et al.,* 2006; Wei *et al.,* 2007, Ruiz-Rodríguez *et al.,* 2009; Xenoulis *et al.,* 2010). En el caso de las aves, ya que la cloaca tiene varias funciones como son eliminar los desechos digestivos, urinarios y el intercambio de gametos, los análisis de desechos digestivos incluyen bacterias asociadas a la última porción de las vías urinarias, y al sistema reproductivo, por lo cual es difícil interpretar los datos (Hupton *et al.,* 2003; White *et al.,* 2010).

Tabla 1. 2: Número de especies bacterianas encontradas a lo largo del tracto gastrointestinal en gallinas y el humano.

Especie	Órgano	No. OTUs	Autor
Gallus gallus	Buche	13	Gong *et al.,* 2007
	Molleja	11	Gong *et al.,* 2007
	Duodeno	14	Gong *et al.,* 2007
	Yeyuno	12	Gong *et al.,* 2007
	Íleon	9 – 72	Lu *et al*., 2003; Gong *et al.,* 2002; 2007
	Ciego	21 – 699	Zhu *et al.,*2002, Gong *et al.,* 2007; Sergeant *et al.,* 2014
Homo sapiens sapiens	Boca	73 – 9600	Paster *et al.,* 2001, Bik *et al.,* 2010; Contreras *et al.,* 2010, Stearns *et al.,* 2011; Belda-Ferre *et al.,* 2012
	Garganta	152	Anderson *et al.,* 2008
	Esófago	85 - 282	Dethlefsen *et al*., 2006, Anderson *et al.* 2008
	Estómago	128 – 657	Bik *et al.,* 2006; Dethlefsen *et al.,* 2006, Anderson *et al.,* 2008, Stearns *et al.,* 2011
	Intestino	2 – 1200	Hayashi *et al.,* 2005, Canny y McCormick, 2008; Rajilić-Stojanović *et al.,* 2007, Zhang *et al.,* 2010
	Heces	301 -1000	Eckburg *et al.,* 2005, Egert *et al.,* 2006, Anderson *et al.,* 2008,

Por lo tanto, el análisis de secuencias bacterianas provenientes de muestras fecales, lavados cloacales o lavados intestinales, podrían no ser representativas de las comunidades bacterianas presentes en el intestino (Zoetendal *et al.,* 2002; Sarva-Rupavtarm *et al.,* 2004; Zoetendal *et al.,* 2006; Wei *et al.,* 2007). De esto se desprende la probabilidad de que la diversidad bacteriana en el TGI podría ser muchas veces mayor que la reportada hasta el momento en la literatura. Por otro lado, las comunidades bacterianas a lo largo del intestino podrían variar en su composición (tanto a nivel de Phyla como de géneros), dependiendo de la región funcional del intestino (lo que involucra cambios morfológicos y de disposición de sustratos; Mai y Morris, 2004; Sarma-Rupavtarm *et al.,* 2004). Por lo tanto, para obtener una muestra representativa de la diversidad bacteriana que habita el ecosistema intestinal, es necesario emplear una mezcla homogénea del intestino (incluyendo tejido intestinal, quimo y heces) o si se desea saber, cuales bacterias habitan en un nicho específico, debería de analizarse cada compartimento por separado.

Comunidades bacterianas y su relación con la dieta*:*

La dieta proporciona un suministro de nutrientes, lo que se traduce en una diversidad de sustratos para las comunidades bacterianas que habitan en el TGI. Mucho se ha discutido acerca de la importancia de la dieta en la conformación de las comunidades bacterianas intestinales, por lo que cabe preguntar ¿Es la dieta el factor más importante en la conformación de las comunidades bacterianas? Y de ser así ¿Cuáles son los mecanismos por los cuales la dieta influye en el tipo de bacterias que se encuentran en el TGI? Todavía se desconocen muchos de los componentes de la relación sustrato – bacteria. Existen tres condiciones básicas que podrían influir en la conformación de las comunidades intestinales dependiendo del tipo y concentración de los sustratos presentes en el ecosistema intestinal: a) sólo las bacterias capaces de metabolizar los sustratos disponibles en el ecosistema estarán presentes (Serikov *et al.,* 2010), b) un sustrato puede derivar en diferentes sustratos, productos de diferentes rutas metabólicas que pueden ser aprovechados por diferentes bacterias (Louis *et al.,* 2006), y c) un mismo sustrato puede ser procesado en diferente formas por diversas bacterias (Macfarlane y Macfarlane, 2003; Louis *et al.,* 2006).

Los cambios en la dieta pueden, por tanto, originar cambios cuantitativos y cualitativos en el suministro de sustratos, presentando diferentes efectos sobre en la composición de las comunidades bacterianas (Louis *et al.,* 2006; Godoy-Vitorino *et al.,* 2012) y tener un efecto directo tanto a corto como a mediano y largo plazo (Gibson y Roberfroit, 2005; Sundset *et al.,* 2007). Se han registrado cambios en la composición de las comunidades bacterianas intestinales asociados a cambios en la dieta en pavos (*Meleagris gallopavo;* Delgado *et al.,* 2006; Scupham *et al.,* 2008), novillos (*Bos taurus*; Delgado *et al.,* 2006), renos (*Rangifer tarandus*; Sundset *et al.,* 2004; 2007) y en el ser humano (*H. sapiens*; Gophna, 2011). En cada uno de estos ejemplos, las comunidades bacterianas están compuestas por los mismos Phyla, pero cambian las proporciones de los diferentes Phyla y/o la composición a nivel de géneros bacterianos (Sundset *et al.,* 2004; Scupham *et al.,* 2008). Estos trabajos arrojan información valiosa de cómo las variaciones en la dieta pueden afectar la composición de las comunidades bacterianas.

Estudios recientes muestran que dependiendo de su dieta, los seres humanos pueden ser clasificados en tres grupos dependiendo de la composición de su microbiota intestinal (enterotipo), cada uno de los cuales

está dominado por uno de los géneros *Bacteroides, Prevotella y Ruminococcus* (Gophna, 2011; Jeffery *et al.,* 2012). Esta clasificación de enterotipos es independiente del sexo, el índice de masa corporal y la nacionalidad. Al combinar un detallado análisis nutricional y el microbioma de 98 individuos, Wu y colaboradores (2011) encontraron que los enterotipos aparentemente están determinados por patrones alimenticios a largo plazo. Así pues, el enterotipo *Bacteroidetes* está asociado con un alto consumo de proteínas animales (De Filippo *et al.,* 2010; Wu *et al.,* 2011), mientras que el enterotipo *Prevotella* está asociado con una dieta rica en material vegetal y carbohidratos con un bajo consumo de carne (De Filippo *et al.,* 2010; Wu *et al.,* 2011). Existe un tercer enterotipo denominado *Ruminococcus*, sin embargo, y de acuerdo con los datos reportados por Wu y colaboradores (2011), este enterotipo no está bien definido y aparentemente se fusiona con el enterotipo Bacteroides.

Estos resultados tienen interesantes implicaciones y plantean la pregunta de si las comunidades bacterianas en hospederos que tienen la misma dieta serán similares, independientemente de su origen filogenético. Hasta el momento sólo existe un trabajo que ha evaluado el efecto de la dieta en la comunidades bacterianas de un gran número de especies de mamíferos con diferentes orígenes filogenéticos (Ley *et al.,* 2008). En este estudio encontraron que especies de mamíferos que tienen la misma dieta, tienen comunidades bacterianas similares, sin importar su origen filogenético. Debido a que la colonización bacteriana del TGI en mamíferos es tanto vertical como horizontal, la dieta puede ser un factor muy importante para determinar las comunidades bacterianas intestinales en este grupo.

De acuerdo con los hábitos alimentarios en los hospederos de cada gremio trófico, se puede esperar que se encuentren bacterias con actividad a) proteolítica en especies carnívoras, b) bacterias recicladoras de nitrógeno especialmente en las aves nectarívoras, (Preest *et al.,* 2003) o en especies que tengan dietas pobres en nitrógeno (Karasawa y Maeda, 1994), c) metanógenas en especies que consumen fibra, d) queratolíticas en especies insectívoras y consumidoras de otros artrópodos, e) bacterias capaces de degradar polisacáridos vegetales en animales granívoros, f) bacterias frecuentes en animales de sangre caliente como *E. coli* y *Clostridium spp.* Finalmente, se podrán encontrar diversas bacterias patógenas como parte de los ecosistemas intestinales de aves y mamíferos (Abulreesh *et al.,* 2007; Lovanh *et al.,* 2007; Benskin *et al.,* 2010). En cuanto a la complejidad de las comunidades intestinales, los resultados del trabajo realizado por Ley y colaboradores

(2008a) muestran que las comunidades bacterianas son más diversas en especies herbívoras, tienen una diversidad intermedia en especies omnívoras, y una baja diversidad en especies carnívoras. A continuación hacemos una breve descripción de los procesos digestivos y de las especies bacterianas que se han encontrado en aves y mamíferos con diversas dietas.

Animales nectarívoros*:* El néctar es una solución diluida de azúcares (principalmente sacarosa, fructuosa y glucosa) en diversas proporciones, con pequeñas concentraciones de aminoácidos, proteínas, lípidos, vitaminas, compuestos secundarios, compuestos orgánicos y minerales (Wäckers *et al.,* 2007; Cronck y Ojeda, 2008). El néctar es un alimento rico en términos energéticos, pero insuficiente para satisfacer los requerimientos de nitrógeno de las aves y mamíferos que se alimentan de él (Nicholson y Thornburg, 2007; Cronck y Ojeda, 2008), por lo que los animales nectarívoros se ven obligados a complementar su dieta con insectos u otra fuente de proteína (Stiles, 1995). El tracto digestivo de los animales nectarívoros es simple y corto (Stevens y Hume, 1998; Klasing, 2005) y los azúcares que conforman el néctar son fácilmente asimilados en el intestino (Martínez del Río y Karasov, 1990; McWhorter *et al.,* 2006). El ambiente intestinal de los animales nectarívoros es rico en carbohidratos, ideal para la colonización bacteriana, por lo que, las comunidades bacterianas del intestino de los nectarívoros podrían ser o relativamente simples, o muy complejas ya que la presencia de carbohidratos como sustrato podría propiciar la presencia de casi cualquier tipo de bacteria (Kersters, 2006; Blaunt y Clavel, 2007). Adicionalmente esperaríamos encontrar en el intestino de los animales nectarívoros especies bacterianas capaces de degradar compuestos nitrogenados, tales como la quitina del exoesqueleto de los insectos, y sobre todo ácido úrico, ya que se ha encontrado la existencia de reflujos de esférulas de este compuesto, producto de la excreción renal de la cloaca al intestino de aves donde se degrada (Braun y Campbell 1989; Preest *et al.,* 2003). Esto permitiría reciclar el nitrógeno, y ayudar con la limitante impuesta por una dieta con bajo contenido de nitrógeno.

Animales frugívoros*:* Los frutos están compuestos en su mayoría de azúcares simples (sacarosa, fructuosa y hexosas), proteínas, fibra (Martínez del Río y Karasov, 1990; Levey y Martínez del Río, 2001) y algunos compuestos indigeribles como xilano, pectina y arabinosa (contenidas en la fibra vegetal; Sonnenburg

et al., 2005; Barry *et al.,* 2010). Las aves y mamíferos pueden digerir con facilidad los azúcares y proteínas contenidas en los frutos (Jordano, 2000; Levey y Martínez del Río, 2001), pero no pueden digerir carbohidratos complejos como las pectinas, celulosa y otros oligosacáridos (Stevens y Hume, 1998; Klasing, 2005; Canny y McCormick, 2008; Barry *et al.,* 2010), carbohidratos que podrían ser metabolizados por la microbiota intestinal. Los animales frugívoros no están adaptados para digerir la fibra y semillas que componen los frutos (Klasing, 2005), aunque muchas especies poseen ciegos alargados que ayudan en la digestión de los compuestos fibrosos de la dieta (Klasing, 2005), sin embargo, algunos autores sugieren que los frugívoros no dependen de los microorganismos para la digestión de la fibra (Gross y Spillman, 2003), debido a que el tiempo que esta permanece en el intestino es muy corto (Klasing, 2005). En el intestino de frugívoros, se espera encontrar un gran número de bacterias capaces de metabolizar diferentes tipos de azúcares, algunas capaces de digerir fibra y otras más que sean proteolíticas. A pesar de la gran diversidad de vertebrados frugívoros, hasta ahora, sólo existe un trabajo que ha evaluado la composición de las comunidades bacterianas en un murciélago frugívoro. Los datos de Klite (1965) muestran que en el intestino del murciélago frutero común (*Carollia perspicillata*), se encontraron miembros de los Phyla Proteobacteria (*Aeromonas, Alcaligenes, Escherichia, Klebsiella, Serratia, Proteus, Pseudomonas*) y Firmicutes (*Bacillus, Clostridium, Enterococcus).*

Animales granívoros*:* Las semillas están compuestas principalmente por carbohidratos (24.0 - 66%), y en menor porcentaje por lípidos (1.5 – 18.5 %), proteínas (0.8 - 7.48%) y, en algunos casos sustancias tóxicas (~01%; Lokesha *et al.,* 1992; Jordano, 2000; Voigt *et al.,* 2004). Las semillas son digeridas con relativa facilidad por las aves, las cuales poseen mollejas grandes, en donde las semillas son reducidas mecánicamente. Adicionalmente las aves granívoras consumen piedras pequeñas para ayudar a este proceso (Koutsos *et al.,* 2001; Klasing, 2005). Estudios hechos en aves granívoras gallinas (*G. gallus*), gansos, pavos (*M. gallopavo*) y pinzones cebra (*Taeniopygia guttata*), muestran que las comunidades bacterianas de granívoras están dominadas por Firmicutes, Actinobacteria, Bacteroidetes y Proteobacteria (Salanitro *et al.,* 1978; Scupham *et al.,* 2008; Benskin *et al.,* 2010; Liu *et al.,* 2011), pudiendo encontrar hasta 685 OTUs (en filogenia: Operational Taxonomic Unit). Es interesante observar que los Phyla bacterianos que dominan las comunidades bacterianas de estas aves son prácticamente los mismos, aunque la proporción de cada uno de ellos varía entre las

especies de hospederos (Lu *et al.,* 2007; Lu y Santo Domingo*,* 2008). Pacheco y colaboradores (2004) realizaron un estudio descriptivo de las comunidades bacterianas en el buche del periquito de rabadilla verde (*Forpus passerinus*), descubriendo que los géneros bacterianos predominantes fueron: *Enterococcus, Lactobacillus, Propionibacterium y Streptococcus,* algunos autores sugieren que estas bacterias pueden degradar el almidón de las semillas (Pacheco *et al.,* 2004). Se espera encontrar en el intestino de estas especies de aves, bacterias capaces de degradar los polisacáridos, lípidos y proteínas que componen las semillas.

Animales insectívoros*:* Muchas de las aves y mamíferos cuya dieta está basada en insectos, tienden a regurgitar, o excretar el exoesqueleto quitinoso de estos (Akaki y Duke, 1999). La degradación de la quitina por vertebrados ha recibido mucha atención debido a que su digestión es baja comparada con la de otros polisacáridos (Pollock, 2002). La quitina es degradada por enzimas quitinolíticas producidas por la mucosa gástrica de muchas especies de vertebrados (Paolletti *et al.,* 2007), sin embargo, existe evidencia de que algunos géneros bacterianos como *Pseudomonas spp, B. chinitivorous y Cytophaga* son capaces de degradar la quitina (Benton, 1934). Hasta el momento sólo se han descrito las comunidades bacterianas en los intestinos del Murciélago Mastín común (*Molossus major,* hoy *Molossus molossus)* y del Murciélago Bigotón de Parnell (*Chilonycteris rubiginosa*, hoy *Pteronotus parnelli;* Herd*,* 1983), ambos murciélagos considerados insectívoros. En el intestino de estos murciélagos también se reportó la presencia de Proteobacteria (*Escherichia*, *Klebsiella-Aerobacter-Serratia, Proteus y Enterobacteria*) y Firmicutes (*Clostridium;* Klite, 1965). Existe solo un trabajo que ha evaluado la diversidad bacteriana en mamíferos insectívoros, usando métodos moleculares, en el tracto gastrointestinal de diversas especies de mamíferos insectívoros (Delsuc *et al.,* 2014). Las comunidades bacterianas en el intestino de nueve especies de mamíferos insectívoros, están conformadas por Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Tenericutes y Verrucomicrobia (Delsuc *et al.,* 2014). Los géneros más abundantes fueron *Prevotella, Streptococcus, Diarista, Klebsiella, Faecalibacterium, Eubacterium y Erysipelotrichaceae* (Delsuc *et al.,* 2014).

Animales carnívoros*:* Como su nombre lo indica, los carnívoros se alimentan de tejido animales. El proceso digestivo de los carnívoros es relativamente simple, ya que la dieta está constituida por un mayor porcentaje de proteínas y bajo porcentaje de carbohidratos (Karasov y Diamond, 1988), ambos relativamente de fácil digestión (Houston y Cooper, 1975). En la digestión ácida, las proteínas son hidrolizadas en el estómago por acción de las pepsinas y HCl, y transformadas en polipéptidos y amino ácidos libres (Randall, 2002). Dado que el estómago de las especies carnívoras suministra las condiciones adecuadas (enzimas proteolíticas estomacales, pancreáticas e intestinales y pH ácido) para la digestión de los músculos y órganos de sus presas (Hilton *et al.,* 1999), se reduce o elimina la necesidad de una comunidad bacteriana compleja que ayude al proceso digestivo. Existen pocos trabajos que hayan descrito las comunidades bacterianas en el intestino de diversos carnívoros, y los resultados indican que existe variabilidad en la dominancia de Phyla de cada una de estas comunidades, aunque los motivos que expliquen esta variación no están claros. Los Phyla dominantes en el intestino de gaviotas (*Larus atricia* y *L. delawarencis*), son Firmicutes, Proteobacteria, Bacteroidetes (Lu *et al*., 2008; Zhang *et al.,* 2014) y Actinobacteria (van Dongen *et al*., 2013; Zhang *et al*., 2014). Mientras que las comunidades bacterianas en el intestino del oso polar (*Ursus maritimus*) está dominada casi exclusivamente por Firmicutes (Glad *et al.,* 2010a). Los Phyla dominantes en el colón de la foca capuchina (*Cystophora cristata*), son Bacteroidetes, Firmicutes y el resto de la comunidad bacteriana está compuesta en menor porcentaje de Proteobacteria y Fusobacteria (Glad *et al.,* 2010b). Finalmente en el pingüino Adelaida (*Pygoscelis adelaie*) se encontró que los Phyla más abundantes son Firmicutes, Actinobacteria, Proteobacteria y Bacteroidetes (Banks *et al.,* 2009). De acuerdo con los resultados de Ley y colaboradores (2008), los mamíferos carnívoros tendrán la menor diversidad de bacterias en comparación con los herbívoros y omnívoros. Los resultados de los trabajos reportados por diversos autores, parecen apoyar esta teoría, ya que se han reportado entre 17 y 190 OTUs (Lu *et al.,* 2008; Glad *et al.,* 2010a; White *et al.,* 2010, van Dongen *et al.,* 2013; Becker *et al.,* 2014; Zhang *et al*., 2014). En especies carnívoras se ha reportado la prevalencia de *Campylobacter, Escherichia/Shigella* y *Salmonella* (Adesiyun *et al.,* 1998), y de las familias Clostridiaceae I, XIVa y XI, Lachnospiracae, Peptostreptococcaceae and Ruminococcaceae (Becker *et al.,* 2014; Zhang *et al.,* 2014).

Animales herbívoros*:* Las especies herbívoras, son definidas como aquellos organismos que pueden subsistir consumiendo una dieta que está compuesta en su mayor parte de material vegetal con un alto contenido de fibra (hojas, peciolos, y ramas; Stevens y Hume, 1998). Existen básicamente dos tipos de herbívoros, aquellos que tienen un estómago compuesto (como el de los rumiantes) y lo utilizan para fermentar, y aquellos que tienen un estómago simple y llevan a cabo la fermentación del material vegetal en ciegos intestinales (Stevens y Hume, 1998; Karasov y Martínez del Río, 2007). Los herbívoros enfrentan diversas dificultades al utilizar material vegetal como fuente primaria de alimento. Cerca del 60% de la biomasa vegetal está compuesta de materiales indigeribles como celulosa, hemicelulosa y lignina (Louis *et al.,* 2006; Karasov y Martínez del Rio, 2007), además de diversos metabolitos secundarios (Kohl *et al.,* 2011). La composición química del material vegetal, puede disminuir la eficiencia digestiva o alterar la homeostasis de los herbívoros (Karasov, 2011; Kohl *et al.,* 2011). A continuación describiremos brevemente ambos tipos de herbívoros y las bacterias que están presenten en su tracto digestivo.

a) Los rumiantes son animales que digieren su alimento en etapas. Primero mastican el alimento, y en una segunda etapa, regurgitan el material semidigerido, y lo vuelven a masticar (Stevens y Hume, 1998). El estómago de los rumiantes está dividido en tres grandes compartimentos multifuncionales (rumen, retículo y omaso) y un compartimento secretorio (abomaso) cuya función es similar a la del estómago de otros vertebrados. El rumen y el retículo funcionan como sitios de fermentación (Stevens y Hume, 1998). Para sobrevivir con una dieta tan pobre de nutrientes y tan difícil de digerir, los rumiantes han establecido estrechas relaciones simbióticas con diversos grupos bacterianos (Wright *et al.,* 2009; Karasov, 2011). El papel principal de estas bacterias involucra la fermentación y digestión de carbohidratos complejos. Las bacterias procesan los polímeros de celulosa por medio de hidrolisis y convertidos en ácidos grasos de cadenas cortas, los cuales son fácilmente absorbidos por el hospedero (Karasov y Martínez del Río, 2007).

Las comunidades bacterianas en el tracto gastrointestinal de los rumiantes han sido estudiadas en varios mamíferos: vacas (*B. taurus*), renos (*Rangifer tarandus*), camellos (*Camelus dromedarius*) y artiodáctilos como gacelas, cebras, toros (*Bos indicus, Equus quagga, Gazella granti, G. rufifrons y Taurotragus oryx*; Nelson *et al.,* 2003; Sundset *et al.,* 2004; 2007; Samsudin *et al.,* 2011) y en el hoatzin (*Opisthocomus hoazin*; Godoy-Vitorino *et al.,* 2008; Godoy-Vitorino *et al.,* 2010; Godoy-Vitorino *et al.,* 2012) . Se han reportado entre 88 y 650

OTUs bacterianas en el intestino de diversos rumiantes (An *et al.,* 2005; Sundset *et al.,* 2007; Godoy-Vitorino *et al.,* 2008; Kong *et al.,* 2010). Estas comunidades bacterianas están dominadas por los Phyla Firmicutes, Bacteroidetes y Archaea (An *et al.,* 2005; Kong *et al.,* 2010), y representantes de los Phyla Actinobacteria, Chloroflexi, Cyanophyta, Cytophaga, Fibrobacter, Lentisphaerae, Planctomycetes, Proteobacteria, Spirochaetes TM7 y SR1 (An *et al.,* 2005; Sundset *et al.,* 2007; Kong *et al.,* 2010; Samsudin *et al.,* 2011). Entre los géneros más abundantes se encuentran *Bacteroides, Prevotella,* (Bacteroidetes)*, Butyrivibrio, Lachnospiraceae, Ruminococcus, Pseudobutyrivibrio (*Firmicutes*) y Fibrobacter* (Fibrobacteres; An *et al.,* 2005; Sundset *et al.,* 2007; Kong *et al.,* 2010). En todas estas comunidades también se ha reportado la presencia de *Methanobrevibacter* spp., grupo de arqueas que metabolizan carbohidratos complejos (Godoy-Vitorino *et al.,* 2008; Wright *et al.,* 2009).

b) Los herbívoros no rumiantes procesan el alimento de manera diferente, ya que tienen una menor capacidad para degradar las paredes de celulosa del material vegetal (Karasov y Martínez del Río, 2007). Los osos panda gigante y el panda rojo (*Ailuropoda melanoleuca* y *Ailurus fulgens*) son un buen ejemplo de este grupo de herbívoros, asimilan menos del 10% de la celulosa y hemicelulosa que ingieren (Karasov y Martínez del Río, 2007). Muchos de estos animales presentan relaciones estrechas con diferentes grupos de microorganismos, en las llamadas cámaras de fermentación localizadas en los ciegos intestinales, dichas cámaras permiten la asimilación de celulosa por acción bacteriana (Karasov y Martínez del Rio, 2007).

Existen pocas descripciones de las comunidades bacterianas en el intestino de herbívoros no rumiantes, estas se han llevado a cabo en el caballo (*Equus ferus*; Daly *et al.,* 2001), el oso panda (*Ailuropoda melanoleuca*; Wei *et al.,* 2007; Kong *et al.,* 2014) y la rata de Bryant (*Neotoma bryanti;* Kohl *et al.,* 2011). Las comunidades bacterianas de herbívoros no rumiantes están compuestas principalmente de Firmicutes, Proteobacteria, Bacteroidetes y Actinobacteria (Daly *et al.,* 2001; Wei *et al.,* 2007; Kohl *et al.,* 2011). El número de OTUs bacterianas oscila entre las 13 y las 477 (Daly *et al.,* 2001; Wei *et al.,* 2007; Kohl *et al.,* 2011; Kong *et al*., 2014). Entre las especies bacterianas más abundantes se encuentran *Eubacterium spp, Butyrivibrio spp, Clostridium spp., Ruminococcus spp*., esta última especie con actividad celulolítica y fibrolitica Daly *et al.,* 2001), *E. coli , Streptococcus* (Wei *et al.,* 2007), *Lactobacillus* (Kohl *et al.,* 2011), *Ruminococcus* (género capaz de transformar polisacáridos vegetales en ácidos grasos de cadena corta), *Coprococcus* (capaz de metabolizar y

utilizar el floroglicinol como sustrato de carbono), así como miembros del Phylum TM7 quienes son conocidos por su capacidad para degradar tolueno (Kohl *et al.,* 2011).

Animales hematófagos*:* Existen sólo tres especies de vertebrados que se alimentan de sangre, y las tres son murciélagos de la familia Phyilostomidae. Chaverri (2006), describió la composición de las comunidades bacterianas del estómago e intestino de 21 individuos de *Desmodus rotuntus* (usando medios de cultivo). Aparentemente el tracto gastrointestinal de los hematófagos es relativamente simple y corto, no existe una diferenciación clara entre intestino delgado y grueso (Gadelha-Alves *et al.,* 2008). Existe la posibilidad, de que al ser de fácil digestión, la sangre pueda ser metabolizada por un gran número de bacterias. El número de especies bacterianas que podrían encontrarse en el tracto gastrointestinal de los hematófagos podría ser muy bajo, dado que la sangre es un alimento que puede ser fácilmente digerido, por ejemplo: la sangre bovina tiene un 21.7% de materia seca la cual está constituida en: 93.1% por proteína, 4.9 % ceniza, 1.0 % grasa y 1.0 % de carbohidratos (Breidenstein, 1982). Hasta ahora, en el estómago e intestino de *D. rotundus* registró la presencia de especies pertenecientes a los Phyla Actinobacteria (*Micrococcus*), Bacteroidetes (*Flavobacterium*), Firmicutes (E*nterococcus; Lactococcus, Staphylococcacus* y *Streptococcus*) y Proteobacteria (*Acinetobacter, Aeromonas, Alcaligenes, Achromobacter, Enterobacter, Escherichia, Hafnia, Flavimonas, Proteus, Serratia* y *Vibrio*). Muchas de estas bacterias se encuentran normalmente en la piel de mamíferos o aves (principalmente estafilococos; Chaverri, 2006), por lo que podrían ser adquiridas de manera accidental por *D. rotundus*, cuando lame la sangre del animal del que se están alimentando.

Animales omnívoros*:* Muchas especies de aves y mamíferos son omnívoras y consumen una amplia variedad de alimentos de origen vegetal y animal, de manera natural la elección del alimento en estas especies está determinada por la combinación de factores como la disponibilidad estacional de alimentos, la eficiencia de forrajeo, los cambios en sus requerimientos nutricionales, la palatabilidad del alimento y los patrones de depredación, aunque en su mayoría los animales omnívoros son oportunistas (Klasing, 2005). Los omnívoros tienen una gran diversidad de estrategias digestivas y adaptaciones para digerir alimentos de diferente origen, por lo que el tracto gastrointestinal es altamente variable en su longitud y complejidad (Klasing, 2005). Existen

pocos trabajos que hayan evaluado la composición de las comunidades bacterianas en el tracto gastrointestinal de aves y mamíferos omnívoros. Uno de esos trabajos fue reportado por Ley y colaboradores (2008), quienes encuentran que el número de especies bacterianas es intermedio al encontrado entre los herbívoros (alta diversidad) y los carnívoros (baja diversidad). Quizá esta diversidad bacteriana intermedia pueda ser explicada por las necesidades digestivas de estas especies, ya que deberían de tener pequeñas poblaciones de especies bacterianas capaces de degradar un gran número de alimentos. Carey y colaboradores (2012) evaluaron las variaciones en la estructura de las comunidades bacterianas en ardillas leopardo (*Ictidomys tridecemlineatus*), durante el ciclo de hibernación. Los resultados de estos autores indican que las comunidades bacterianas en *I. tridecemlineatus* están conformadas básicamente por los Phyla Bacteroidetes, Firmicutes y Verrucomicrobia. Los porcentajes de Bacteroidetes y Verrucaria aumentan (ambos Phyla tienen especies capaces de sobrevivir de sustratos derivados del hospedero como las mucinas) mientras que el porcentaje de Firmicutes disminuye (prefieren los polisacáridos presentes en la dieta) durante el período de hibernación (Carey *et al*., 2012). El hombre es uno de los omnívoros mejor estudiados. Los resultados de diversos trabajos muestran que en el intestino de los seres humanos se encuentran miembros de los Phyla Actinobacteria, Bacteroidetes, Chlamydiae, Cyanobacteria, Deferribacteres, Deinococcus-Thermus, Firmicutes, Fusobacteria, Proteobacteria, Spirochaetes, SR1, TMT, y Verrucomicrobia (Dethlefsen *et al.,* 2007; Bik , 2009). El número de OTUs reportado para el tracto gastrointestinal del ser humano oscila entre las 2 y las 9600 (Paster *et al.,* 2001; Anderson *et al.,* 2008; Bik, 2009; Bik *et al.,* 2010; Contreras *et al.,* 2010; Stearns *et al.,* 2011; Belda-Ferre *et al.,* 2012). Los géneros bacterianos más frecuentes son: *Escherichia*, *Streptococcus, Prevotella, Veillonella, Helicobacter* (Bik, 2009), entre otras.

Como se mencionó anteriormente, existen pocos trabajos que describan las comunidades bacterianas en el tracto gastrointestinal en aves omnívoras como avestruces (*Sruthio camelus*), cuervos (*C. macrorhynchus*), emús (*Dromaius novaehollandiae*) y gansos, los resultados de estos trabajos reportan entre 46 y 822 OTUs (Liu *et al*., 2011; Bennett *et al.,* 2013; Maeda *et al.,* 2013), los géneros más abundantes fueron *Apicomplexa, Bacteroides, Clostridia, Eimeria, Erysipelotrichi, Helicobacter*, y *Campylobacter* (Liu *et al*., 2011; Maeda *et al.,* 2013).

A pesar de que los resultados de todos los trabajos presentados en esta revisión indican que la

diversidad de bacterias en el ecosistema gastrointestinal es mayor de lo esperado, sin embargo, aún debe explorarse no sólo su diversidad genética, taxonómica y funcional, sino la distribución geográfica (anatómica) de estas comunidades bacterianas.

Consideraciones finales ¿Determina la dieta la composición de las comunidades bacterianas?

Mucho se habla del papel funcional que las bacterias tienen en el ecosistema presente en el TGI, es importante considerar que algunas de las bacterias que se encuentran en las comunidades bacterianas del intestino de aves y mamíferos, más que ser miembros funcionales del ecosistema intestinal (Edwards *et al.,* 2004; Lovanh *et al.,* 2007), son adquiridas por ingestión accidental (Stewart y Rambo, 2000); tomadas del ambiente o heredadas de la familia (Banks *et al.,* 2009). Algunas bacterias de los géneros *Aquabacterium, Actinomycetes* y *Cyanobacteria (*géneros asociados con material vegetal, o que habitan en agua y suelo) pueden ser ingeridas de manera accidental (Ley *et al.,* 2006; Banks *et al.,* 2009). Sin embargo, cuando estas bacterias no esperadas en el TGI son abundantes, debemos suponer que se encuentran jugando un papel dentro del sistema, independientemente de la forma en que lograron colonizarlo.

Como se puede apreciar en esta revisión, la dieta tiene un efecto importante en la composición de las comunidades bacterianas en el TGI de aves y mamíferos. Más allá de ayudar a metabolizar los componentes de la dieta, las bacterias intestinales pueden tener un papel fundamental en la elección que el hospedero hace de su dieta (Norris *et al.,* 2012). Existe evidencia de que tanto la dieta del hospedero (Nelson *et al.,* 2003; Sundset *et al.,* 2004; 2007; Ley *et al.,* 2008) como la filogenia del hospedero pueden determinar la composición de las comunidades bacterianas (Nelson *et al.,* 2003; 2012; Sundset *et al.,* 2004; 2007). Ley y colaboradores (2008) llevaron a cabo un vasto estudio donde compararon la composición del microbioma intestinal de 60 especies de mamíferos. La composición de las comunidades bacterianas parece diferir entre los diferentes hospederos, dependiendo en algunos casos, de la dieta y de la conformación del intestino. Mientras que para otras especies, las relaciones filogenéticas del hospedero influyen sobre la conformación de las comunidades bacterianas presentes en el intestino. Estos autores, argumentan que el tamaño de su muestra es relativamente pequeño (número de especies y de individuos) para poder dilucidar la influencia relativa de la dieta, la filogenia y la

morfología intestinal en la estructuración de las comunidades intestinales (Ley *et al.,* 2008).

El dilucidar cuál es el factor más importante en la conformación de las comunidades bacterianas del TGI tiene importantes consecuencias para entender la evolución de procesos digestivos, e incluso comprender competencia por recursos. Los hospederos cuyas comunidades bacterianas están adaptadas a explotar un mayor número y tipos de recursos, podría tener una mayor ventaja cuando compite por recursos con especies con una microbiota "pobre" (Ruiz-Rodríguez *et al.,* 2009). También podría explicar, la repartición de recursos en una comunidad compuesta por especies filogenéticamente distantes que explotan recursos similares (Ruiz-Rodríguez *et al.,* 2009). Para dilucidar el efecto que la dieta tiene en la composición de las comunidades bacterianas, debemos de encontrar un modelo adecuado de estudio. Así pues, las aves nectarívoras tienen numerosas cualidades que las hacen un excelente modelo de estudio, en nuestro país existen aves nectarívoras en diversas familias (Emberizidae, Thraupidae, Trochilidae); algunas de ellas tienen la misma distribución geográfica y se alimentan de las mismas flores. Este último punto es quizá uno de los más interesantes, ya que el néctar es la dieta más simple del planeta (compuesta en su mayoría de una mezcla de diversas azúcares con bajas cantidades de proteína y minerales), y por lo tanto tiende a ser bastante homogénea en comparación con la dieta de otras aves y mamíferos.

Por otro lado, es importante además de evaluar el papel que la dieta tiene en la conformación de las comunidades bacterianas, describir la riqueza y diversidad de bacterias a lo largo de del TGI, determinar cuál es el papel funcional que tiene cada una de las especies bacterianas que componen el ecosistema intestinal y relacionar las características del bioma intestinal y co-relacionarlo con las características ecológicas del hospedero.

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Research Article

Gut Bacterial Diversity of the House Sparrow (*Passer domesticus*) Inferred by 16S rRNA Sequence Analysis

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Abstract Intestinal bacteria play an important role in animal health. They extract and process nutrients present in their host's diet, help to develop their host's immune system, and recycle organic compounds, water, and minerals. The gut bacterial diversity is poorly known in wild animals. This study is the first description of the diversity of bacteria along the whole intestine of a wild bird (*Passer domesticus*). Pyrosequencing of the 16S rRNA gene unveiled a high bacterial diversity, distributed in 11 bacterial phyla. The most abundant groups were Proteobacteria and Firmicutes. Bacterial diversity was greater in the upper section of the intestine and decreased toward the final portion of the gut. After a conservative denoising of the sequences, we found 4,436 OTUs in the gut of *P. domesticus*. Our data shows that the diversity of intestinal bacteria in the gut of wild birds is much larger than what had previously been estimated using fecal samples.

Keywords intestinal bacterial communities; bacterial diversity; pyrosequencing; 16S rRNA; House Sparrow

1 Introduction

The gastrointestinal tract of animals is a complex ecosystem influenced both by the intrinsic characteristics of the animal's gut, and the ensemble of bacteria, Archaea, protozoa, and fungi that dwell in it [30, 63]. All these microorganisms are present in immense numbers and are collectively known as the gut microbiota [5, 74]. The microbiota is involved in many functions like synthesis of vitamins [41, 52, 59], enzymatic digestion [30, 33, 57], nutrient, salt and water recycling [46, 63], and activation of the host's immune system [2, 26, 68]. Despite the importance of bacteria in the gut microbiota, little is known about its diversity and functional role inside intestinal ecosystems of wild animals [44, 45, 59].

The study of intestinal bacteria has been limited by our capacity to cultivate them under laboratory conditions [65]. This problem was partly circumvented by the use of molecular approaches like the sequencing of 16S rRNA genes [6, 34, 76], which allows the identification of the bacteria present in different environmental samples [27, 67, 77]. In the last decade, many authors have used 16S rRNA genes to describe the bacterial diversity in the gut of some mammals [16, 33, 45, 50, 53, 66, 77], while bacterial diversity in wild birds has been described mostly using Ribosomal Intergenic Spacer Analysis (RISA) [6, 39, 72]. These studies suggest the existence of a large bacterial diversity in the gastrointestinal tract of mammals and birds, with the principal bacterial phyla being Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and an uncultivated group known as TM7 [34, 53, 77].

Given the complex structure and diverse functional roles of the gut (digestion, nutrient recycling, waste production, etc.), it is crucial to obtain samples from the whole gastrointestinal tract to understand the real dimension of gut bacterial diversity. Additionally to bacterial diversity, it is important to know the functional role of the bacteria in each region of the gut. The difficulty, and ethical implications related to sampling the different intestinal sections has resulted in the frequent use of fecal matter as an indicator of the intestinal microbiota. However, gut bacteria are poorly represented in fecal matter [15, 59, 71, 77], limiting the information that fecal material provides on the bacteria present in the gut mucosa [15, 59, 77], and does not allow for a description of changes in bacterial communities along the different sections of the gut. It can be assumed that

microbiota changes along the intestinal tract because of the biochemical and morphological differences that can be found in the different sections of vertebrate intestines [41, 59]. Thus the main objectives of this study were to describe the bacterial intestinal communities of House Sparrows (*Passer domesticus*) captured in the wild, and to portray the existence of changes in the bacterial communities along the whole length of the intestine, including the cloaca. This is the first work to describe the changes in bacterial communities in the different sections of the intestine of a wild bird using molecular methods.

2 Material and methods

Our work was conducted with permission from the National Institute of Ecology (INE), Mexico, and approved by Animal Care and Use Committee of Ecosystems Research Center (CIEco), National Autonomous University of Mexico (UNAM). Sample size was reduced due to ethical considerations related to the need to sacrifice wild birds to conduct our study.

2.1 Sample collection

We explored the intestinal bacterial diversity by sampling the whole intestines of two female individuals of *P. domesticus*. Birds were captured using mist nest in the gardens of CIEco, UNAM, campus Morelia, located in West Mexico (19°39′00″ north latitude and 101°14′00″ west longitude). To avoid contamination from bacteria present in the bird's food, we kept the two captured birds in cages for two and a half hours without food prior to tissue collection. Because gut-passage times in this species are long for a bird of its size (120–140 min) [9], this procedure allowed us to ensure that the intestines were empty at the moment of tissue collection. Birds were euthanized with ether and their intestines were immediately removed, dissected, and frozen in liquid nitrogen while the tissue was still fresh (birds were euthanized in conformity with Mexican laws and the codes of practice included in the guidelines for the use of wild birds in research). To ensure that no changes in bacterial communities occurred after the bird's death, the whole tissue collection protocol was conducted in less than five minutes. Intestines were divided and kept in a sterile phosphate buffer solution (Na_2HPO_4 0.1 M and NaH_2PO_4 0.1 M, pH 7.4) and stored in a laboratory at −70 °C until processing.

Because it is not possible to identify each intestinal section (ileum, jejunum, duodenum, and hind gut) without help of a stereoscopy, and doing a correct morphological identification takes time and implies conducting several cuts in the tissue [30, 55, 57], we decided to divide the intestine in three fractions of similar size (Upper section—the section contiguous to the stomach: Up; medium section: Md; and Lower section—the final section containing the cloaca: Lw). By doing so, we reduced the possibility of contamination of our samples by external bacteria, and limited the changes in the intestinal bacterial communities that occur due to the modifications of the intestinal ecosystem that follow tissue death. While the three sections of the intestine we analyzed do not correspond directly with the intestine functional parts, they allowed us to describe changes in microbial communities along a gradient of intestinal function where digestion and absorption of nutrients decreases, and water absorption and waste management increases toward the cloaca [30, 57, 63]. While the first two sections of the intestine (Up and Md) represented portions of the small intestine, the last one (Lw) included a part of the small intestine, the large intestine, and the cloaca due to the small size of both the large intestine and the cloaca in this species.

2.2 DNA extraction from gut samples

We extracted DNA using a modification of the method proposed by Nordgård et al. [51]. Briefly, each intestinal region was dissected longitudinally, washed, and macerated with a plastic pestle in saline solution (0.85% NaCl and 0.1% Tween 80) to remove unattached bacteria from the gut walls, and to collect the bacteria in the intestinal content. The upper phases were collected. This process was repeated for three cycles followed by low speed centrifugation (4,500 rpm for 5 min) to remove the larger particulate matter. Upper phases were collected and centrifuged at 12,000 rpm (15 min). The supernatant was eliminated and the pellet was resuspended in 1 mL of TE buffer (10 mM Tris-Cl and 5 mM EDTA pH 8) containing β-mercaptoethanol (5 μL/mL). The samples were frozen and thawed, alternating between 5 min immersion in liquid nitrogen and 8 min at 65 °C for five cycles, and then centrifuged to separate broken and unbroken cells. The pellet (with the unbroken cells) was eluted in 0.5 mL of lysis buffer (0.2 M NaOH, 2 mg/mL lysozyme, and 1% SDS), macerated, and kept at room temperature for 5 min. This process was repeated twice. Washes containing both lysed and suspended cells were combined after the DNA extraction and 1 μL of RNase was added. This was followed by three repeated extractions with phenol-chlorophorm-isoamyl alcohol (25:24:1) and the DNA was precipitated with ice-cold 96% ethanol [49]. All samples were stored at −70 °C until the PCR amplification step.

2.3 Amplification of 16S rRNA and pyrosequencing

16S rRNA genes were amplified in a first PCR reaction with a forward primer corresponding to nucleotide position 8–27 of *Escherichia coli* (Bact-8F 5′-AGAGTTTGATCMTGGCTAG-3′) and a reverse primer corresponding to complement positions 1510-1489 (U-1510R 5′-TACGGYTACCTTGTTACGACTT-3′). A 25 μL reaction volume contained 12.8 μL of water, 1 μL of mix dNTPs (10 mM, Invitrogen), 1 μL of each primer

Table 1: Bacterial species richness (OTUs) in the different sections of the intestine of the House Sparrow (all sequences for each sample). Comparison was conducted using the same number of sequences (1,152). Because the different sections of the intestine share bacterial taxa at all the different taxonomic levels, numbers of OTUs in each section do not add to the total number of OTUs for the whole intestine. Up: upper section, Md: medium section, and Lw: lower section for individual 1 (PD1) and 2 (PD2). Data was obtained using a threshold of 97%.

	PD1Up	PD1Md	PD1Lw	PD2Up	PD2Md	PD2Lw	PD1	PD2
Barcode	CAAGGTTC	CACACACA	CACTACTC	CACTTGAG	CAGAGACA	CATCTGGA		
Total data for each sample								
Sequences	43,048	10,377	6,042	11,897	7,103	1,152	59,467	20,152
Phyla	8	6	4	11	6	4	8	11
Class	15	12	8	20	12	7	17	
Orden	40	27	18	38	23	9	41	50
Families	90	55	30	81	50	15	100	105
Genus	323	148	68	209	138	33	367	248
OTUs Cd-hit	3,158	1,013	281	808	506	93	4,436	1,404
OTUs Mothur	3,187	1,088	267	813	565	99	4,430	1,545
OTUs RDP	5,480	1,815	668	1,246	634	101	7,589	2,639
At 1,152 sequences								
Sequences	1,152	1,152	1,152	1,152	1,152	1,152		
Phyla	5	4	3	7	4	4		
Class	8	7	6	11	8	7		
Orden	18	13	9	23	16	9		
Families	34	26	18	37	29	15		
Genus	100	61	35	71	68	32		
OTUs RDP	451	395	269	334	268	101		

(10 pmol/μL), 5 μL of 10$\times$ buffer, 2 μL of $MgSO_4$ 50 mM, 0.2 μL of Taq polymerase (Platinum Taq DNA Polymerase High Fidelity kit from Invitrogen), and 2 μL of DNA. The following PCR cycling conditions were used: 96 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 60 °C for 30 s, 68 °C for 35 s and a final extension at 68 °C for 10 min. This was followed by a secondary PCR reaction using the primers 16S 27F (5′-AGAGTTTGATCMTGGCTAG-3′) and 16S 533-R (5′-TTACCGCGGCTGCTGGCAC-3′) in which a different barcode primer was used for each sample (Table 1) [25], using the same PCR conditions. Finally, the PCR products were purified using the MinElute PCR Kit (Qiagen). Adapters were added after the libraries were constructed. Pyrosequencing was performed using a Roche FLX GS-Titanium at the National Laboratory of Genomics and Biodiversity, Cinvestav, Mexico.

2.4 Sequence analysis

Each one of the three intestinal sections was analyzed separately. Sequences below 200 bp were removed from the analysis. AmpliconNoise was used to eliminate the effect of sequencing errors, PCR single base substitutions and PCR chimeras [54]. Sequences were clustered in Operational Taxonomic Units (OTUs at 97% of DNA sequence identity), which is the consensus threshold for species boundaries [75], using CD-Hit [35], the pipeline of RDP [13], and Mothur [60]. We reported the number of OTUs from our samples obtained by these three algorithms. For taxonomic assignment, the partial sequences of 16S rRNA genes corresponding to the *E. coli* gene position 7–500 were analyzed using the RDP's Pyrosequencing Pipeline [70] with an 80% confidence threshold. All sequences analyzed belonged to the Bacterial Division, and we were able to assign most of them to genera, for which assignment reliability was estimated to be over 97% [36].

We used rarefaction analysis, from the RDP pipeline, to compare bacterial species richness between individuals and among intestinal sections. This analysis computes species accumulation curves based on the repeated re-sampling of all clusters. Rarefaction curves represent the statistical expectation for observed accumulation curves [24], enabling the comparison of the statistically expected species richness of each community at the same sampling effort or abundance [48].

Because our sampling methods allowed us to detect changes in bacterial diversity along the gut, we decided to use analytic methods from the field of community ecology to compare the structure of the bacterial communities from the different sections of the House Sparrow gut. To do this, we used Whittaker plots [40], also known as rank/abundance plots. This method describes communities of organisms based on the abundance of the different taxonomical groups present in a community. The shape of the linear relationship between abundance and rank of the taxa represents different models of resource usage [40], and its slope indicates the level of dominance among different communities [40]. To compare the rank/abundance plots of the bacterial communities, we used analysis of covariance (ANCOVA).

Because we were unable to identify enough bacteria at the species level to compare among intestine sections, we conducted our analysis at the level of genus. We compared the bacterial communities in the gut of *P. domesticus* at two levels: for individual (gathering all sequences) and between intestinal sections of individuals. We compared among sections of the gut to determine if bacterial communities changed along the gut. To compare among intestinal sections, and due to the fact that each sample had a different number of sequences, we used the minimal number of sequences present in a sample, and randomly, selected the same number of sequences from the other samples. We expected bacterial communities to be simpler and more dominated toward the end of gut, because the diversity and abundance of nutrients becomes reduced toward the cloacal region [30, 63]. Finally, we compared the bacterial diversity of the same intestinal section of the two House Sparrows.

Bacterial diversity and species richness were estimated by the Chao1 Index using the program EstimateS [13] included in the pipeline of RDP. Chao1 estimates total species richness as follows:

$$\text{Chao1} = S_{\text{obs}} + \frac{n_1^2}{2n_2},$$

where S_{obs} is the number of observed species, n_1 is the number of singletons (species captured once), and n_2 is the number of doubletons (species captured twice) [12]. This index is useful for data sets skewed toward the low-abundance classes, as in the case of intestinal bacterial [28].

Another measure of α-diversity is the Shannon Index. We estimated this index using the program EstimateS [13] included in the pipeline of RDP. The index is expressed as follows:

$$H' = \sum_{i=1}^{S} p_i \log_2 p_i,$$

where S is the number of species, p_i is the proportion of individuals of the specie i with respect to all individuals (relative abundance of specie i: n_i/N), n_i is the number of individuals of specie i, and N is the number of individuals of all species [14].

Additionally to α-diversity, we used β-diversity (the partitioning of biological diversity among environments or along gradients, e.g., the number of species shared between two environments) [48]. We compared the beta diversity between individuals and between intestinal sections using a presence/absence dissimilarity index (Simpson β_{SIM}). This index is given by the formula

$$\beta_{\text{SIM}} = \frac{A \min B}{C + A},$$

where A corresponds to the number of shared genera between two samples, B and C represent the number of unique genera in the sample one and two, respectively, while "min" is the minimum value of restricted genera in the compared samples. This index shows maximum values (high diversity) when the percentage of shared genera is low, and the percentage of gains/losses between gut sections are similar [18, 31]. Because β_{SIM} is an index of dissimilarity, we reported our results as $1 - \beta_{\text{SIM}}$ to show similarity.

The sequences obtained were deposited in the MG-Rast server ID 4521283.3. All of them were deposited in a unique file, and every sample was labeled as PD1Up, PD1Md, PD1Lw, PD2Up, PD2Md, and PD2Lw before the ID of each sequence.

3 Results

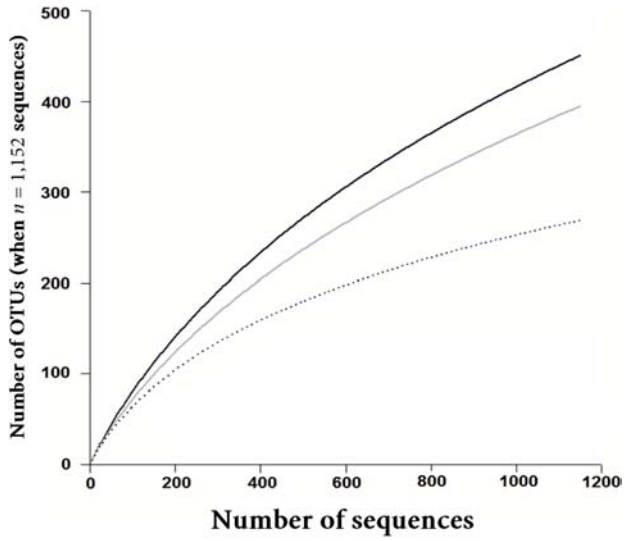
3.1 Bacterial diversity at the individual level

The number of total sequences varied between the two birds sampled. For individual 1 (PD1) we obtained a total of 59,467 sequences, while for individual 2 (PD2) the number was lower (20,152, Table 1). The Cd-Hit analysis showed 4,436 and 1,404 OTUs for PD1 and PD2, respectively. The analysis of the RDP indicated that the sequences of PD1 were grouped in 7,589 OTUs, while in the individual 2, we found 2,639 OTUs. Finally the Mothur analysis showed that the number of OTUs were 4,430 for PD1 and 1,545 for PD2 (Table 1). Rarefaction analysis showed that the bacterial species richness for the two individuals at the same number of sequences was higher in PD1 than in PD2 (Figures 1(a) and 1(b)).

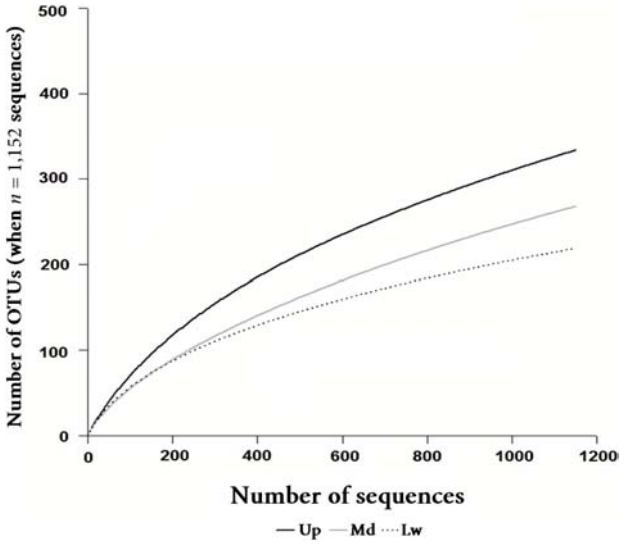
The analysis from the RDP's pipeline showed that the House Sparrow gut presented 11 bacterial phyla: Acidobacteria, Actinobacteria, Aquificae, Bacteroidetes, Chlorobi, Cloroflexi, Cyanobacteria/Chloroplast, Deinococcus-Thermus, Firmicutes, Planctomycetes, and Proteobacteria. In both individuals, gut bacterial communities were dominated by Firmicutes and Proteobacteria (between 48.02% and 48.97%, and between 60.44% and 36.56% of all sequences, resp., for PD1 and PD2; Figure 2). Other phyla were present in smaller numbers (representing between 3.01% and 3.02% of all sequences).

We found that the number of families identified for each individual was higher in PD2 than in PD1 (105 and 100 bacterial families, resp.; Table 1). We found that the gut of *P. domesticus* was dominated by the same bacterial families in both individuals: Propionibacteriaceae (Phylum Actinobacteria); Carnobacteriaceae, Lactobacillaceae, and Streptococcaceae (Phylum Firmicutes); and Burkholderiales_incertae_sedis, Comamonadaceae, Enterococcaceae, Enterobacteriaceae, Moraxellaceae, Pseudomonadaceae and Sphingomonadaceae (Phylum Proteobacteria). The vast majority of the bacterial families were represented only by a few bacterial sequences.

Finally, the pipeline's RDP analysis identified a total of 367 genera of bacteria for individual 1 and 248 for



(a)



(b)

Figure 1: Rarefaction curves for bacterial species richness in the gut of House Sparrow at a cut point of 1,152 sequences. Comparison of OTUs richness for the three gut sections for (a) individual 1 (PD1) and (b) individual 2 (PD2); Up: upper section, Md: medium section, and Lw: lower section. Data was obtained using a threshold of 97%.

individual 2 (Appendix 1). The most abundant genera were *Aquabacterium*, *Atopostipes*, *Catellicoccus*, *Dolosigranulum*, *Escherichia/Shigella*, *Lactobacillus*, *Lactococcus*, *Lactovum*, *Pelomonas*, *Sphingomonas*, *Paralactobacillus*, *Rhizobacter*, *Sphingosinicella*, *Streptococcus*, and *Sphingopyxis*. The Chao1 indicator showed that the number of bacterial OTUs that we found in the gut of both individuals was lower than the expected value ($13,068.01 \pm 499.36$ (PD1) and $4,936.6 \pm 346.81$ (PD2)), indicating that the

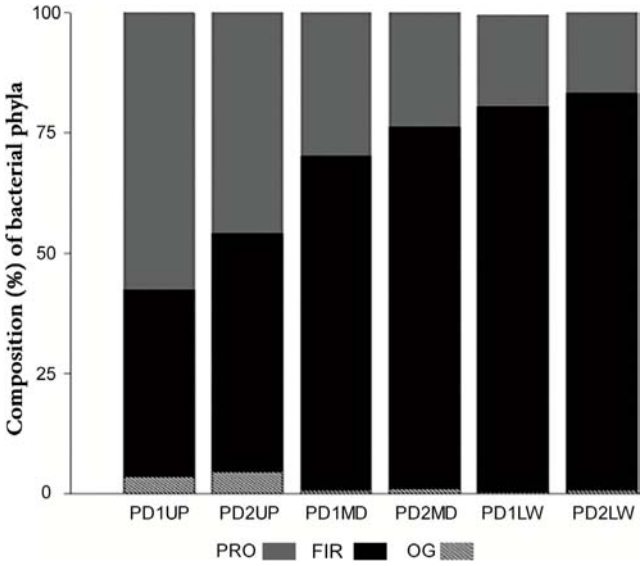


Figure 2: Percent composition of different Phyla along the gut of the House Sparrow. FIR: Firmicutes, PRO: Proteobacteria, and OG: Other groups (including the rest of phyla that composed the bacterial communities in the gut of House Sparrow). Data was obtained using a threshold of 97%.

bacterial diversity in the gut of the House Sparrow should be even higher than the numbers we reported.

The value of the Shannon Index for PD1 was 7.431; while for the individual 2 it was 6.2955. These values indicate that the bacterial communities of the gut of both individuals had a high bacterial diversity. The bacterial communities are constituted by a large number of species with low abundances (Figure 4).

3.2 Changes in bacterial diversity along the gut

Our data showed that the number of sequences and OTUs varied among the three intestinal sections of the gut. The number of OTUs for each intestinal section was variable for each individual (in both analyses, Table 1). We summarized the information on bacterial diversity for all the different taxonomic levels in Table 1.

Because the number of species that can be found in a sample increases with sample size [40], and since we obtained different numbers of sequences for the different sections of the gut of our two House Sparrow individuals, we used a common method to balance our sample size in order to conduct appropriate comparisons among the different intestinal sections [40]. We used the number of sequences from the section with the lowest number of sequences as our baseline sample size (1,152 sequences from PD2Lw). Then, we randomly selected the same number of sequences from each one of the other gut sections. These procedures allowed us to have an identical sequence size, and bypass any bias caused by differences in sequence number [40].

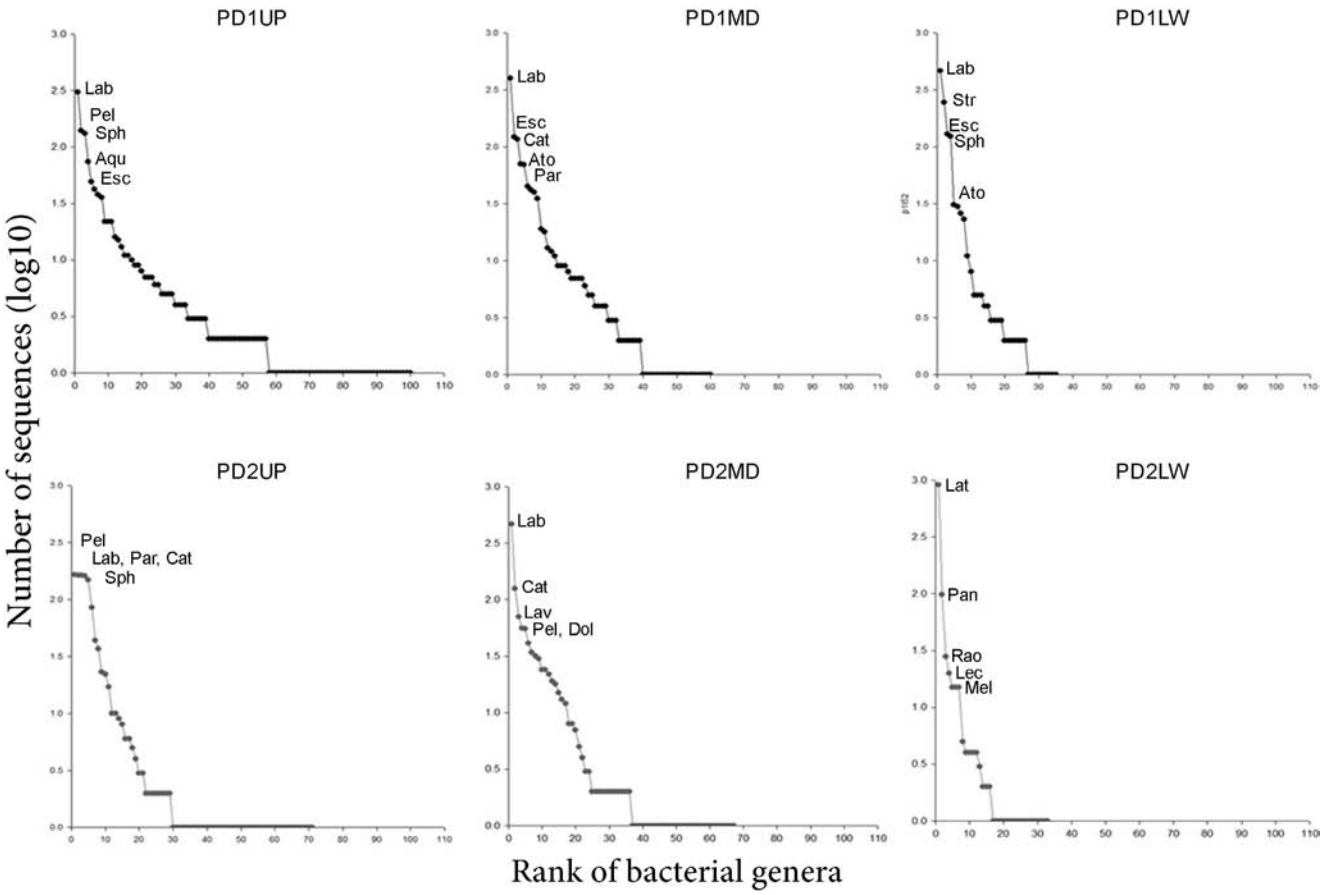


Figure 3: Rank-abundance curves of bacterial genera present in the different sections of the gut of the House sparrow. Distribution of the abundance of the different bacterial genera for the three gut sections for (a) individual 1 (PD1) and (b) individual 2 (PD2). Most common bacterial genera were Aqu.—*Aquabacterium*, Ato.—*Atopostipes*, Cat.—*Catellicoccus*, Dol.—*Dolosigranulum*, Esc.—*Escherichia/Shigella*, Lab.—*Lactobacillus*, Lat.—*Lactococcus*, Lav.—*Lactovum*, Lec.—*Leclercia*, Mel.—*Melissococcus*, Pan.—*Pantoea*, Par.—*Paralactobacillus*, Pel.—*Pelomonas*, Rao.—*Raoultella*, Sph.—*Sphingomonas*, Str.—*Streptococcus*. Data was obtained using a threshold of 97%.

When we compared the bacterial diversity of the different sections of the gut, we found a clear reduction of diversity toward the final portion of the gut (Table 1). The comparison of the number of OTUs at the same number of sequences among sections of the gut showed that the total number of bacterial species differed in each of the intestinal sections ($P < .05$; 44; Figures 1(a) and 1(b)) [39]. We also found that the number of bacterial genera was larger in the first section of the gut, intermediate in the middle section, and lower in the final section for both individuals (Figures 3(a) and 3(b)).

The RDP's pipeline indicated that the intestinal communities of the gut were composed basically by Firmicutes and Proteobacteria, and a large collection of phyla were poorly represented (Figure 2). Together, the two main phyla made up to 95.85% and 99.65% of all the bacteria in all intestinal sections for individuals 1 and 2, respectively. The percentage of Proteobacteria was higher in the first section of the gut, and decreased toward the end of the gut, while the percentage of Firmicutes was lower at the gut's beginning, and increased toward the end of the gut in both individuals (Figure 2). Bacterial communities were similar along the different sections of the intestine, with the most abundant bacterial genera being present in all the sections of the intestine. The same genera dominated the bacterial communities along the gut, with the exception of the lower section of individual 2, which had bacterial communities with a composition that differed (Figure 3).

When we analyzed bacterial community structure using the rank/abundance curves, we found that in each intestinal section of the gut the bacterial communities of both individuals were dominated by a small number of genera represented by a high number of sequences, while the great majority of the genera were represented only by few sequences (Figures 3(a) and 3(b)). We found that 16 bacterial genera dominated the different sections of the

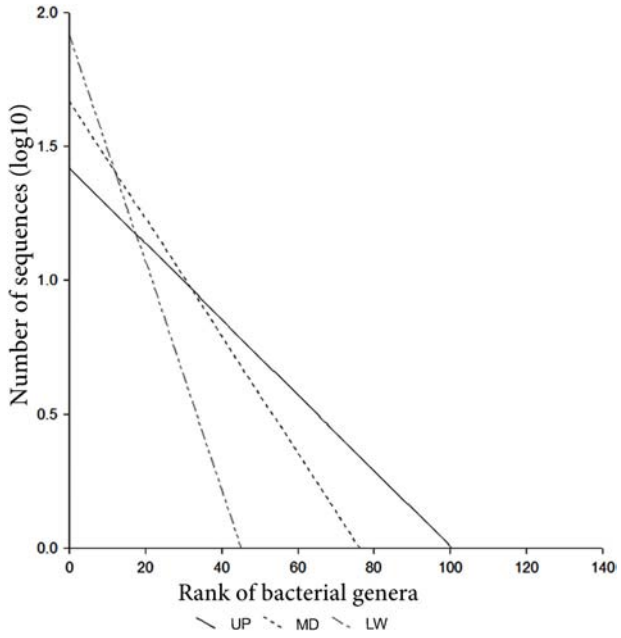


Figure 4: Bacterial communities for the different sections of the gut of the House Sparrow show differences in their values of dominance/evenness. The most diverse and less dominated communities were present in the upper intestinal section, and the less diverse and more dominated communities were found in the lower section. The differences in the structure of bacterial communities can be explained by the availability of food resources along the intestinal ecosystem. Data was obtained using a threshold of 97%.

gut (Figures 3(a) and 3(b)), with the number of bacterial genera decreasing toward the cloaca. Bacterial communities were complex, diverse, and less dominated in the first intestinal section, becoming simpler, less diverse, and more dominated in the middle and lower intestinal sections (Figure 4), suggesting that the bacterial diversity in the gut of this bird depends on the diversity and abundance of nutrients (Table 2).

When we compared the same intestinal sections between the two individuals, we found notable differences. For the upper section of the gut, the number of Phyla, Orden, and Families were larger in individual 2 than in individual 1 (Table 1), but the number of genera and OTUs were larger in individual 1 (100 vs. 71 genus, and 451 vs. 334 OTUs, resp., for individuals 1 and 2). The middle section of the intestine was similar in the number of Phyla, Orden, Families, and genera in both individuals, with the number of OTUs being larger in individual 1 (395 and 268 OTUs, resp., for individuals 1 and 2, with a similarity of 53%). Finally, the lower intestinal portion was similar in the number of Phyla, Order, families, and genera in both individuals (269 and 101 OTUs for individuals 1 and 2, resp.), with a similarity of 73%. The most common bacterial genera in each one of the

Table 2: Similarity ($1-(\beta_{\text{SIM}})$) of bacterial communities at genera level among the different sections of the gut, and between individuals. Comparisons conducted at the same number of sequences (1,152). Data was obtained with a threshold of 97%.

Comparisons	$1-(\beta_{\text{SIM}})$
Between sections	
PD1UP vs. PD2UP	0.5493
PD1MD vs. PD2MD	0.5254
PD1LW vs. PD2LW	0.7333
Along the gut	
PD1UP vs. PD1MD	0.7299
PD1MD vs. PD1LW	0.7059
PD1UP vs. PD1LW	0.8065
PD2UP vs. PD2MD	0.7172
PD2MD vs. PD2LW	0.8
PD2UP vs. PD2LW	0.8068

Table 3: Comparisons of bacterial community dominance for each intestinal section combining data from both individuals. We described bacterial community structure at the genera level. Data obtained with a threshold of 97%.

Comparison	**ANCOVA**
Up vs. Md	$F_{1,228} = 4.0447$, $P = .0447$
Md vs. Lw	$F_{1,151} = 129.7396$, $P =< .0001$

intestinal sections of the House Sparrow gut are presented in Figures 3(a) and 3(b). The comparison along the gut sections using β_{SIM} showed that the bacterial communities of contiguous gut sections (upper and middle, and middle and lower) were more similar than those of not adjacent sections (first and final; Table 3). β_{SIM} index showed that bacterial communities gain few bacterial families along the gut, with this pattern occurring in both individuals (Table 2).

4 Discussion

Our results show that the number of bacterial species present in the gut of the House Sparrow is large with 4,436 species-level OTUs (from the CD-Hit analysis). Our study described the presence of a higher number of bacteria in the gut of a wild bird than those reported by previous studies for the gut of birds, some mammals, and even humans [16, 22, 32, 34, 62]. Because the analysis we used not only eliminated sequences that include chimera, homopolymer sequences, and sequences created by errors of pyrosequencing [56], but also it removed some real bacterial sequences by its stringent criteria, we expect the total number of OTUs present in the House Sparrow's gut to be higher. Thus, the number of OTUs we report here is a conservative estimate of the bacterial diversity living in the gut of our study species. Even though our results are a demure estimate of the bacterial diversity present in the intestinal ecosystem of birds, our data conclusively shows that intestinal bacterial diversity in birds is much larger than previously thought [2, 74, 77].

4.1 Bacterial diversity in the House Sparrow's gut

How can we explain this high bacterial diversity in the gut of a small granivorous bird? We believe that our capacity to make a better description of the gut bacterial communities of birds is related to the fact that we sampled the whole intestine, instead of using only fecal matter or cloacal swabs like in previous studies [15,39,58,71]. In humans, for instance, it has been shown that the bacterial composition obtained from gut biopsies and fecal samples from the same individuals can vary enormously [15].

Because to sample the whole gut is technically complex, and/or requires sacrificing organisms, the use of fecal matter and cloacal swabs is the most common technique used to describe intestinal bacteria diversity in vertebrates [59, 74,77]. Previous studies that used fecal matter, or cloacal swabs, reported numbers of OTUs for the gut of mammals and wild birds that are lower than our findings (between 93 and 281 OTUs). These numbers are similar to those we found in the last section of the intestine of the House Sparrow [23]. This suggests that the use of feces or cloacal swabs could only be detecting the bacteria present in the final portion of the gut instead of providing a good estimate of the whole gut bacterial communities.

The methods that we used to process our gut samples and extract DNA also could have played a critical role in allowing us to detect a higher bacterial diversity than previous studies. Inside the intestinal environment, bacteria species can get established as biofilm communities on the surface of the intestinal wall intimately attached to epithelial tissue, deeply embedded in the mucus layer overlaying the villi, free-living in the lumen, or colonizing the crypts and plica at the base of the villi [1,42,47,55]. By using a homogenate of the complete intestine before extracting the DNA, we ensured the presence of bacteria from all the intestinal habitats.

4.2 Bacterial composition along the House Sparrow's gut

The bacterial communities present in the intestine of various animals are composed of at least 17 phyla of Bacteria [16, 34,77]. Most of the gut bacterial communities described for mammals are dominated by Firmicutes and Bacteroidetes, and a low percentage of other phyla [16,34,62]. In birds, the dominant bacterial phyla are similar to those of other animals. Chicken guts are dominated by Firmicutes [62,76], while turkeys' intestinal communities can be dominated either by Firmicutes [37] or Bacteroidetes [62]. In the few wild birds that have been studied, bacterial communities were dominated by Firmicutes; however the second dominant phylum varied: Actinobacteria in Adelaide Penguins and Proteobacteria in Zebra-Finches [6,8]. In the Hoatzin (*Ophistocomus hoatzin*) a folivorous bird with crop microbial fermentation [20], the microbial community of the crop was dominated by Firmicutes and Bacteroidetes [20,21,22] while the bacterial communities of the foregut were dominated by Bacteroidetes and Actinobacteria, and contained a lower proportion of Firmicutes and Proteobacteria [21].

In our study, we found that the gut bacterial communities of the House Sparrow were dominated by Firmicutes and Proteobacteria in similar numbers. Together these two groups comprise over 96% of all bacterial sequences in the complete gut. The dominance of Firmicutes and Proteobacteria in the intestinal bacterial communities has been reported for other birds like Zebra Finches [8], as well as wild and captive parrots [73]. Because Proteobacteria is a group with a high functional diversity [29], the dominance of Proteobacteria in the House Sparrow gut could be related to its omnivorous diet that includes seeds, insects, fruits, and a large diversity of human food scraps including meat [9, 11,49]. This diverse diet provides bacterial communities with a high diversity of substrates.

The dominance of Firmicutes and Proteobacteria in the gut of the House Sparrow changed along the intestine. We found that in the first section there was a greater proportion of Proteobacteria, with this group decreasing its relative abundance toward the final region of the intestine, where Firmicutes represented a higher percentage of the sequences. While other studies indicate that the bacterial intestinal communities were dominated by Firmicutes (in the range of 22.6–100%) [3,20] and Bacteroidetes (in the range of 1.1–56%) [19,65], our results show that Firmicutes is the dominant bacterial phylum in the second and third sections of the gut of House Sparrows (Figure 2).

We also found that the bacterial community composition differed among gut sections (Figures 2(a), 3(a), and 3(b)). We believe that this is the consequence of changes in nutrient diversity and abundance, and the different habitat conditions present along the gut. Within the intestine there is a gradient of diminishing nutrient availability with changes in pH, oxygen levels and rates of peristaltic movement [30, 57,63]. While some of the habitat characteristics (like pH or oxygen levels) could have important effects on the bacterial communities, the reduction in the diversity and availability of metabolizable substrates along the gut is probably the reason for the decreasing diversity of bacterial genera and species along the gut of the House Sparrow (Figures 3(a) and 3(b)) [41,55,59]. Previous studies of the physiology of this species showed that the activity of intestinal enzymes decreased along the gut as a response of a reduction of nutrients [9].

4.3 Functional role of bacteria in the gut

Bacteria are one of the most diverse groups of organisms taxonomically and functionally [10,27,56]. Bacteria have a great ability to metabolize various substrates and to change their metabolism depending on the substrates present in their environments [27,37,38,56,77]. For these reasons, it

is difficult to assign a defined functional role to the different groups of bacteria that we found in the intestinal ecosystem of the House Sparrow based only on the description of its bacterial communities unless a metagenomic study is performed [7].

Firmicutes is one of the most diverse and abundant phylum within the gastrointestinal tract of vertebrates. They include a wide variety of uncultured organisms, and it has been reported that some members of this phylum are able to degrade starch and cellulose [4]. Because the functional role of the majority of the Firmicutes is unknown, it is difficult to hypothesize the activities they execute in the intestinal ecosystem [77]. However, the genus *Lactobacillus* in the family Lactobacillaceae has been well studied in the past [37, 69, 77].

We found that *Lactobacillus* was one of most abundant bacterial genera in the House Sparrows' gut (Figures 3(a) and 3(b)). The high abundance of *Lactobacillus* could be related to the capacity of these bacteria to metabolize starch, the main sugar present in the seed that this species eats [52]. Starch is a highly insoluble complex carbohydrate with an indeterminate molecular weight [52]. Lactobacilli can degrade starch into maltotriose, maltose, and glucose. These oligosaccharide and disaccharide are hydrolyzed to monosaccharides by enzymes located in the microvilli of intestinal cells, while the monosaccharide glucose is absorbed or transported directly to the blood [57, 63]. Although fermentation of starch produces less energy than its conversion to glucose by endogenous enzymes, microbial fermentation could be an advantage to animals on a high-starch diet [52]. While we did not sample the crop and stomach of the House Sparrow, our results indicate that the number of sequences on the genus *Lactobacillus* diminished toward the end of the intestine. This reduction in *Lactobacillus* abundance seems to be associated to the fact that most of the sugar digestion and absorption occurs in the first section of the intestine, limiting the amount of sugars that bacteria can use in the rest of the gut [43, 61].

The other well-represented group in the gut of House Sparrows is the Proteobacteria. This phylum is the largest bacterial group, and represents an extremely diverse group, both at morphological and physiological levels [56]. However, the role of the Proteobacteria in the gut of birds is unknown and remains to be explored.

The presence of different bacterial species in the intestinal ecosystem does not guarantee that the hosts obtain a direct benefit from them, or that the bacteria are part of the intestinal ecosystem. It is often difficult to determine whether or not a particular microorganism is truly autochthonous to a particular host and provides it with some benefits [17, 69]. It is probable that some of the bacteria we found in the gut of the House Sparrow were only "hitchhikers" or originally acquired by accidental ingestion [64], inherited as heirlooms, or acquired as accidental souvenirs [6, 64]. In our study case, we supposed that some intestinal bacteria are "hitchhiking" like Aquabacterium, Actinomycetes, and Cyanobacteria. However, most of the bacteria we found must be a part of the intestinal ecosystem, because they were represented by a relatively large number of sequences, and because we ensured that the intestines were empty before we collected our tissue samples.

Our study presents the first descriptive study of the composition of the bacterial communities that inhabit the whole intestine of a wild bird. It helps to understand the high diversity of bacteria present in the intestine of birds, and how this diversity changes along the different sections of the gut. Our results show that the bacterial diversity in the gut of a granivorous bird could be larger than 4,000 OTUs, and that bacterial diversity decreases toward the end of the intestine, providing evidence that the bacterial communities in the gut are more diverse than previously anticipated, and opening new courses of action to study the diversity and functional role of bacteria in the intestinal ecosystem.

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Capítulo 3

Gut bacterial diversity of two nectarivorous birds with different phylogenetic origins (*Cynanthus latirostris* – Trochilidae, *and Diglossa baritula* - Emberizidae) inferred by 16S rRNA sequence analysis

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Abstract

There is little information regarding the phylogenetic, functional and metabolic diversity of microbial species that inhabit the gastrointestinal tract of vertebrates. Despite the important role that the bacterial communities of the gastrointestinal tract have on the fitness of their hosts, the factors that influence the shaping of these bacterial communities are poorly known. Strong evidence indicates that diet is the most important factor determining the composition of bacterial communities in vertebrates. However vertical transmission of bacteria from parents to offspring could generate a phylogenetic signal in gut bacterial communities. In order to determine the relative role that diet and phylogeny play on the formation of gut bacterial communities in birds, we described the bacterial communities of two species of nectarivorous birds that have different phylogenetic origins, a hummingbird (Broad-billed Hummingbird – *Cynanthus latirostris*, family Trochilidae, order Apodiformes) and a Flowerpiercer (Cinnamon-billed Flowerpiercer - *Diglossa baritula*, family Emberizidae, order Passeriformes). We compared our results from these species with the bacterial communities of another passerine, the House

sparrow (*Passer domesticus*, family Passeridae, order Passeriformes). We found 7129 and 9621 OTUs in the gut of *C. latirostris* and *D. baritula* respectively, belonging to 20 Phyla. The gut bacterial communities of *C. latirostris* and *D. baritula* were similar in composition sharing 72.1% of bacterial genera. We also observed that the bacterial diversity was lower in *P. domesticus* than in the two nectarivorous species. The dominant bacterial Phyla were Proteobacteria, Firmicutes and Actinobacteria. All three species shared between 70.9 and 76.5 % of the bacterial genera, but our data show significant differences in the composition of their bacterial communities. The most abundant genera in nectar-feeding birds were: *Acinetobacter, Buttiauxella, Enterobacter, Pelomonas, Ralstonia, Sphingomonas and Streptobacillus*. A clustering analysis shows that although the three bird species share several bacterial genera, bacterial communities found in the gut of nectar-feeding birds share a higher similarity between them, than with those present in the gut of the House Sparrow. Our results suggest that diet may be the most important factor to determine the composition of gut bacterial communities.

Key words: Intestinal bacterial communities, bacterial diversity, pyrosequencing, 16S rRNA, *Cynanthus latirostris, Diglossa baritula, Passer domesticus*.

Introduction

At present, there is little information regarding the phylogenetic, functional and metabolic diversity of the microbial species that inhabit the gastrointestinal tracts of vertebrates (Breitbart *et al.,* 2003; Zoetendal *et al.,* 2006). However, recent studies have shown that gut bacterial communities of vertebrates are complex, and include a diverse array of at least 17 bacterial phyla (Scupham *et al.,* 2008; Ley *et al.,* 2008; Godoy-Vittorino *et al.,* 2010). Despite the importance that gut bacterial communities have on the fitness of their host, the factors that regulate the conformation of these bacterial communities are poorly known (Stevens and Hume, 1998; Ley *et al.,* 2008). Bacteria colonize the gut of vertebrates before birth (DeReu *et al*., 2006; Ardissone *et al*., 2014; Cacho and Neu, 2014; Moles *et al*., 2014), and continue after birth by a diversity of pathways, that include parental care and feeding, contact with the environment, and diet (Stevens and Hume, 1998; Newburg 2000; O'Hara and Shanahan, 2006). Whatever the way in which the bacteria are acquired, factors intrinsic and extrinsic to the host determine the final community of bacteria that is found in its gastrointestinal tract (Stevens and Hume 1996; Lu *et al.,* 2003a; Dethlefsen *et al.,* 2007).

Different studies suggest that the colonization of the gastrointestinal tract by bacteria is affected by intrinsic characteristics of the host like gut morphology (Miron *et al.,* 2001; Ley *et al.,* 2008), age (Lu *et al.,* 2003a; Zoetendal *et al.,* 2008; Godoy-Vittorio *et al.,* 2010), genetic composition (Zoetendal *et al.,* 2001; Lu *et al.,* 2003b; Ley *et al.,* 2006; Scupham *et al.,* 2008) and phylogenetic relationships (Maul *et al.,* 2005; Ley *et al.,* 2008; Glad *et al.,* 2010). While some other studies show that the composition of bacterial communities is regulated by extrinsic factors, like geographic location of the host (Sunset *et al.,* 2007) and the composition of the diet (Sunset *et al.,* 2004, 2007; Kong *et al.,* 2010).

There is strong evidence that indicates that diet composition is the most important factor shaping the composition of bacterial communities in vertebrates. Nelson *et al.* (2003) showed that the bacterial communities found in the gut of five ruminant species belonging to two different Orders were very similar, despite their phylogenetic relationships. Also they found that the differences in the bacterial communities can be explained by small differences in the hosts' diets (Nelson *et al.,* 2003). However, Ley and collaborators (2008) found that both host diet, and phylogeny, seemed to influence bacterial diversity in mammals. They sampled the bacterial communities of 59 mammalian species, and found that species that have similar diets cluster together. Additionally, hosts that belong to the same Orden also clustered. However, their results are not conclusive, because in mammals, species belonging to the same Order usually have similar diets.

In mammals a phylogenetic effect can be limited because only some bacteria are obtained by the offspring before, during and after birth (Blaser, 2006; Palmer *et al.,* 2007; Zilber-Rossenber and Rosenberg 2008; Ardissone *et al.*, 2004; Cacho y Neu, 2014; Moles *et al*., 2014), of by feeding from the mother's milk (Newburg, 2000; O'Hara and Shavahan, 2006; Palmer *et al.,* 2007), the saliva of mother (Newburg, 2000; Dethlefsen *et al.,* 2007; Palmer *et al.,* 2007), contact with other individuals of the same species (Newburg, 2000; Zilber-Rossenber and Rosenberg, 2008), and by coprophagia (Stevens and Hume, 1998), limiting the vertical transmission of bacterial communities. However, in birds we can expect to find a different pattern because nestlings acquire bacteria during the incubation period, the bacteria may be transferred directly from the mother from direct contact with the egg during the incubation period (Potter *et al*., 2013). The bacteria may have an environmental origin, but some evidence suggest that bacteria can enter eggs before and after they are laid (De Reu *et al.,* 2006), allowing for a vertical transmission of some gastrointestinal bacteria when the egg passes

through the mother's cloaca; the next step in the bacterial colonization occurs when chicks are fed by the parents. The chicks acquired most of their intestinal microbes from the regurgitated food that adults feed directly to their crops and/or stomachs (Salanitro *et al.,* 1978; Mills *et al.,* 1999), and for direct contact with the parent's cloaca and feces in the nest (Hebb *et al.,* 2003; Maul *et al.,* 2005; De Reu *et al*., 2006).

In order to determine the role that diet and phylogeny play on the composition of bacterial communities in birds, we compared the bacterial communities of two species of nectarivorous birds that have no close phylogenetic relationship, a hummingbird (Broad-billed Hummingbird – *Cynanthus latirostris*, family Trochilidae, order Apodiformes) and a Flowerpiercer (Cinnamon-billed Flowerpiercer - *Diglossa baritula*, family Emberizidae, order Passeriformes), and compared them to the bacterial communities of the House sparrow (*Passer domesticus*, family Passeridae, order Passeriformes). This study is the first to describe the intestinal bacterial communities of wild nectarivorous birds, describing changes in bacterial community composition along their guts. Furthermore, since the diet of nectar-feeding birds is relatively simple, we can expect that bacterial communities inhabiting the gut of these birds should be less diverse and more dominated than the bacterial communities present in gut of a granivorous bird (House Sparrow).

Methods

Our work was conducted with permission from the National Institute of Ecology (INE), Mexico, and approved by Animal Care and Use Committee of Ecosystems Research Center (CIEco), National Autonomous University of Mexico (UNAM). Sample size was reduced due to ethical considerations related to the need to sacrifice wild birds to conduct our study.

Sample collection

We explored the intestinal bacterial diversity by sampling the whole intestines of one individual of Broad-billed Hummingbird (*Cynanthus latirostris)* and two individuals of Cinnamon-billed Flowerpiercers (*Diglossa baritula).* Birds were captured using mist-nest in Chiquimitio, Michoacán (*C. latirostris*; 19°48'00" North and 101º 15' 00" West), and Estación Biológica las Joyas (19° 34' 14" North and 104º 19' 49" West) and Nevado de Colima (*Diglossa baritula*; 19° 33' 49" North and 103º 36' 29" West). Birds were euthanized with ether and their

intestines were immediately removed, dissected and frozen in liquid nitrogen. Birds were euthanized conforming to Mexican laws and codes of practice, and following the guidelines for using wild birds in research (Fair *et al*., 2010).

Intestines were divided and kept in a phosphate buffer solution (Na_2HPO_4 0.1M and NaH_2PO_4 0.1M, pH 7.4) and stored in a laboratory at -70ºC until processing. Because it is not possible to identify each intestinal section (ileum, jejunum, duodenum and hind gut) without help of a stereoscopy, and doing a correct morphological identification takes time and implies conducting several cuts in the tissue, we decided to divide the intestine in three fractions of similar size (Knarreborg *et al.,* 2002; Rabiu and Gibson, 2002; Klasing, 2005; Mirón *et al.,* 2014). By doing so, we reduced the possibility of contamination by external bacteria, and/or the presence of changes in the intestinal bacterial communities due to modifications of the intestinal ecosystem following tissue death. While the three sections of the intestine we analyzed do not correspond directly with its functional parts, they allowed us to describe changes in microbial communities associated with a gradient of intestinal function, digestion and absorption of nutrients decreases, and water and management of waste increases towards the cloaca (Stevens and Hume 1998; Klasing 2005. While the first two sections of the intestine (Upper and Middle) represented portions of the small intestine, the last one (Lower) included a part of the small intestine, the large intestine, and the cloaca due to the small size of both the large intestine and the cloaca in the studied species. This allowed us to have enough tissue to conduct our bacterial DNA extraction protocol. Data for the third species (House Sparrow; *Passer domesticus*) was taken from a previous study conducted using conducted by our research group using exactly the same methodology (Mirón *et al.,* 2014). By adding this species to our data set, we were able to compare gut bacterial communities at two levels: 1) a phylogenetic level by comparing two species from the same Order with different diets (*Passer domesticus* - granivorous, and *Diglossa baritula* - nectarivorous), and 2) a diet level by comparing two species from different Orders with the same diet (*Cynanthus latirostris* - Order Apodiformes, and *D. baritula* - Order Passeriformes). These comparisons were conducted both for the whole gut of each species, and for each gut section. Detailed data on the gut bacterial communities of the House sparrow can be consulted in the original publication (Mirón *et al.,* 2014).

DNA extraction from gut samples

We extracted DNA using the method proposed by Nordgård and collaborators (2005) with some modifications. Briefly, each intestinal region was opened longitudinally, washed and macerated with a plastic pistil in saline solution (0.85% and 0.1% Tween 80) to remove unattached bacteria from the gut walls, and for collecting the bacteria in the intestinal content. This process was repeated for three cycles followed by low speed centrifugation (450 rpm for 5 min) to remove the larger particulate matter. Upper phases were collected and centrifuged at 1200 rpm (15 min). The pellet was resuspended in 1 ml of TE buffer (10 mMTris-Cl and 5 mM EDTA pH 8) containing β-mercaptoethanol (5 µl/ml). Samples were frozen and thawed, alternating between 5 min immersion in liquid nitrogen and 8 min at 65°C for five cycles, and then centrifuged to separate broken and unbroken cells. The pellet was eluted in 0.5 ml of lysis buffer (0.2 M NaOH, 2 mg/ml lysozyme and 1% SDS), macerated, and kept at room temperature for 5 min. This process was repeated twice. Both cells (lysed and intact suspended) were combined after the DNA extraction and 1µl of RNAse was added. This was followed by three repeated extractions with phenol-chlorophorm-isoamyl alcohol (25:24:1) and the DNA was precipitated with ice-cold 96% ethanol (Norgard *et al.,* 2005). All samples were stored at -70ºC until the PCR amplification step.

Amplification of 16S rRNA and pyrosequencing:

16S rRNA genes were amplified in a first PCR reaction with a forward primer corresponding to nucleotide position 8-27 of *Escherichia coli (*Bact-8F 5'-AGAGTTTGATCMTGGCTAG-3') and a reverse primer corresponding to complement positions 1510-1489 (U-1510R 5'-TACGGYTACCTTGTTACGACTT). Routinely, 25 µl reactions contained 12.8 µl of water, 5 µl of 10x buffer, 1µl of mix dNTPs (10mM Invitrogen), 2 µl of MgSO4 50 mM, 1µl of each primer (10 pmol/µl), 0.2µl of Taq polymerase (Platinum Taq DNA Polymerase High Fidelity Invitrogen) and 2 µl of DNA. The following PCR cycling conditions were used: 96°C for 2 min, followed by 30 cycles of 95°C for 30 secs, 50°C for 30 secs, 72°C for 60 secs and a final extension at 68°C for 10 min. This was followed by a second PCR reaction using the primers 16S 27F-5'-AGAGTTTGATCMTGGCTAG-3' and 16S 533-R TTACCGCGGCTGCTGGCAC in which a different barcode primer was used for each sample (Table 3.1; Hamady *et al.,* 2008), at the same PCR conditions. Finally the PCR products were purified using the MinElute PCR Kit (Qiagen). Pyrosequencing was performed using a Roche FLX GS-Titanium at the National Laboratory

of Genomics and Biodiversity, CINVESTAV (Mexico).

Sequence analysis

Each one of the intestinal sections of the two nectarivorous birds was analyzed separately. Sequences below 200 bp were removed from the analysis. AmpliconNoise was used to eliminate the effect of sequencing errors, PCR single base substitutions and PCR chimeras (Wang *et al.,* 2007). Sequences were clustered in Operational Taxonomic Units (OTUs at 97% of DNA sequence identity), which is the consensus threshold for species boundaries the pipeline of RDP (Cole *et al.,* 2011). For taxonomic assignment, the partial sequences of 16S rRNA genes corresponding to the *E. coli* gene position 7–500 were analyzed using the RDP's Pyrosequencing Pipeline (Wang *et al.,* 2007) with an 80% confidence threshold. All sequences analyzed belonged to the Bacterial Division, and we were able to identify most of them to the genera level with, assignment reliability of over 97% (Liu *et al.,* 2007).

We used rarefaction analysis, from the RDP pipeline, to compare bacterial species richness between species and among the different intestinal sections. This analysis computes species accumulation curves based on the repeated resampling of all clusters. Rarefaction curves represent the statistical expectation for observed accumulation curves (Gotelli and Colwell, 2001) enabling the comparison of the statistically expected species richness of each community at the same sampling effort or abundance (Moreno and Halffter, 2001).

Because our sampling methods allowed us to detect changes in bacterial diversity along the gut, we decided to use analytic methods from the field of community ecology to compare the structure of the bacterial communities from the different gut sections of the two species of nectarivorous birds. To do this, we used Whittaker plots (Magurran, 2004), also known as rank/abundance plots. This method describes communities of organisms based on the abundance of the different taxonomical groups present in a community. The shape of the linear relationship between abundance and rank of the taxa represents different models of resource usage (Magurran, 2004), and its slope indicates the level of dominance among different communities (Magurran, 2004). To compare the rank/abundance plots of the bacterial communities, we used analysis of covariance (ANCOVA).

Due to the size of our genetic marker we were able to do our analysis at the genus level (at 97% of DNA sequence identity). We compared the bacterial communities in the gut of our study species in two ways: for each

species (gathering all sequences), and between intestinal sections of individuals. We compared among sections of the gut to determine if bacterial communities changed along the gut, and to determine how different these changes between our two study species were. To compare among intestinal sections, and due to the fact that each sample had a different number of sequences, we used the minimal number of sequences present in a sample, and randomly, selected the same number of sequences from the other samples. We expected bacterial communities to be simpler and more dominated toward the end of gut, because the diversity and abundance of nutrients becomes reduced toward the cloacal region (Stevens and Hume, 1998; Klasing, 2005). Finally, we compared the bacterial diversity of the same intestinal section of the two species of nectarivorous birds.

Bacterial diversity and genera richness were estimated using Chao1 Index in the program EstimateS (Cole *et al.,* 2014) included in the RDP pipeline. Chao1 estimates total species richness as follows:

$$Chao1 = S_{obs} + \frac{n1^2}{2n_2}$$

where S_{obs} is the number of observed species, n1 is the number of singletons (species detected once), and n2 is the number of doubletons (species detected twice; Chao, 1984). This index is useful for data sets skewed toward the low-abundance classes, as in the case of intestinal bacterial (Hughes *et al*., 2001).

Another measure of α-diversity is the Shannon Index. We estimated this index using the program EstimateS (Cole *et al.,* 2014) included in the RDP pipeline. The index is expressed as follows:

$$H' = \sum_{i=1}^{S} p_i \, log_2 p_i$$

where S is the number of species, pi is the proportion of individuals of the specie *i* with respect to all individuals (relative abundance of specie *i*: ni/N), *ni* is the number of individuals of specie *i*, and N is the number of individuals of all species (Crist *et al.,* 2003).

Additionally to α-diversity, we used β-diversity (the partitioning of biological diversity among environments or along a gradients, e.g., the number of species shared between two environments; Moreno and Halffter, 2001). We compared the beta diversity between individuals and between intestinal sections using a presence/absence dissimilarity index (Simpson β_{SIM}). This index is given by the formula:

$$\beta_{SIM} = \frac{A \min B}{C + A}$$

Where A corresponds to the number of shared genera between two samples, B and C represent the number of unique genera in the sample one and two respectively, while "min" is the minimum value of restricted genera in the compared samples. This index shows maximum values (high diversity) when the percentage of shared genera is low and the percentage of gains/losses between gut sections are similar (Moreno and Halffter, 2001; Koleff *et al.,* 2003). This index shows maximum values (high diversity) when the percentage of shared families is low and the percentage of gains/losses between gut sections were similar). Because β_{SIM} is an index of dissimilarity, we reported our results as 1- β_{SIM} to show similarity (Koleff *et al.,* 2003; Gaston *et al.,* 2007).

Additionally we conducted a Bray-Curtis multivariate clustering analysis to evaluate and graphically present, in the form of a dendrogram, levels of similarity among the bacterial communities for the two nectarivorous bird species and the House sparrow. This analysis was performed using Biodiversity Pro (Mcaleece 1997).

All the sequences generated by this study were deposited in MG-Rast with the following acquisition numbers: for all sequences for all individual Cyla 4550681.3, Diba1 4550882.3 and Pado1_cd 4550683.3.

Results

Bacterial diversity at the species level

The number of total sequences varied between the birds sampled. For *Cynanthus latirostris* we obtained a total of 52,711 sequences, while for *Diglossa baritula* we obtained 53,996 sequences (Table 3.1). The analysis of RDP indicates that the sequences of *C. latirostris* were grouped in 10,293 OTUs, while in *D. baritula* we found 9,621 (Table 3.1).

Table 3.1. Composition of bacterial communities in the intestine of *Cynanthus latirostris* (Cyla), *Diglossa baritula* (Diba) and *Passer domesticus* (Pado). Data obtained with a threshold of 97%.

Sample	No. Sequences	Phyla	Orden	Families	Genera	OTUs	Chao	Shannon
All gut sections								
Cyla	52713	17	69	169	616	10293	10624.23 ± 70.13	8.02
Diba	53996	18	63	152	557	9621	18223.78 ± 660.86	7.75
For intestinal section								
CylaUp	21083	11	51	37	445	4448	8566.50 ± 465.46	7.23
CylaMd	1298	9	23	51	129	752	1663.23 ± 229.28	6.34
CylaLw	30332	14	61	236	480	4771	8122.40 ± 377.44	7.08
DibaUp	11274	11	44	100	333	3874	8126.50 ± 504.49	7.57
DibaMd	20551	11	47	99	365	4406	8339.48 ± 502.14	7.32
DibaLw	22173	13	56	362	363	4458	8809.71 ± 483.54	7.20
For comparisons by gut section between nectar-feeding birds								
Cylaup	11274	9	47	118	360	2675	2738.39 ± 24.03	7.02
Cylafn	11274	14	51	115	352	2646	2966.09 ± 59.96	6.90
Dibaup	11274	12	44	101	342	3874	8126.50 ± 504.49	7.57
Dibamd	11274	12	45	101	291	2163	2242.13 ± 23.97	6.55
Dibafn	11274	11	36	85	274	2210	2269.61 ± 20.08	6.16
Between Wildbird Species (at 52713 sequences)								
Cyla	52713	17	69	169	616	10293	10624.23 ± 70.13	8.02
Diba	52713	17	64	158	549	8582	8722.56 ± 42.01	7.66
Pado	52713	10	51	121	397	7082	7188.95 ± 36.99	7.53

Cyla: *Cynanthus latirostris*, Diba: *Diglossa baritula*, Pado: *Passer domesticus*. Up: upper section, Md: media section and Lw: lower section that includes cloaca and ceca

The analysis from the RDP's pipeline showed that the gut of C. latirostris presents 17 Phyla (Table 3.1): Acidobacteria, Actinobacteria, Aquificae, Bacteroidetes, Chlamydiae, Chloroflexi, Cyanobacteria/Chloroplast, Deinoccocus-Thermus, Firmicutes, Fusobacteria, Lentisphaerae, Planctomycetes, Proteobacteria, Spirochaetes, Synergistetes and Thermotogae (Table 3.2). We also found some members of phylum Euryarcheota (Archaeas; Table 3.2). The most important phyla present in the gut of the hummingbird were Proteobacteria (70.63% of all sequences), Fusobacteria (8.5%), Actinobacteria (7.96%), Aquabacteria (7.96%), and Bacteroidetes (3.41%), representing together 99.54% of the total sequences present in this species (Fig. 3.1).

Table 3.2. Composition per Phyla of bacterial communities in the intestine of *Cynanthus latirostris* (Cyla), *Diglossa baritula* (Diba) and *Passer domesticus* (Pado) at different number of sequences. Data obtained with a threshold of 97%.

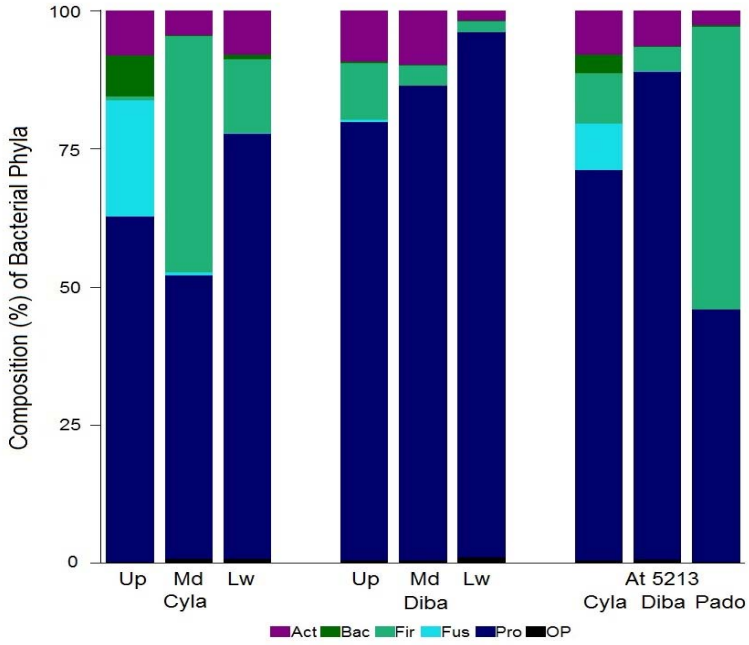
Phyla	**Cyla**	**Diba**	**Cylaup**	**Cylafn**	**Dibaup**	**Dibamd**	**Dibalw**	**Diba**	**Pado**
Acidobacteria	12	30		6	2	2	14	28	8
Actinobacteria	4195	3430	860	854	1037	191	1108	3361	1379
Aquificae	3	1		1	1			1	
Bacteroidetes	1797	138	818	94	31	27	25	131	167
Chlamydiae	1	1						1	
Chlorobi		1			1			1	
Chloroflexi	2	2		1					2
Cyanobacteria/Chloroplast	72	215	4	24	13	82	28	210	13
Deferribacteres		1				2		1	
Deinococcus-Thermus	1			1					3
Euryarcheota	98	12	2	39	5	3	1	12	
Firmicutes	4770	2348	100	1487	1162	224	11274	2291	26962
Fusobacteria	4480	69	2373	20	48	9	1	68	
Lentisphaerae	1	2				1		2	
Planctomycetes	4	13				6	1	11	11
Proteobacteria	37229	47678	7115	8732	8949	10713	9668	46540	24167
Spirochaetes	22	26		8		16		26	
Synergistetes	1		1						
Tenericutes	25	28	1	6	23		2	28	1
Thermotogae		1			1				
Number of sequences	52713	53996	11274	11274	11274	11274	11274	53713	52713

Cyla: *Cynanthus latirostris*, Diba: *Diglossa baritula*, Pado: *Passer domesticus*. Up: upper section, Md: media section and Lw: lower section that includes cloaca and ceca.

The gut bacterial communities of *D. baritula* were composed by 16 phyla: Acidobacteria, Actinobacteria, Aquificae, Bacteroidetes, Chlamydiae, Chlorobi, Chloroflexi, Cyanobacteria/Chloroplast, Deferribacteres, Firmicutes, Fusobacteria, Lentisphaerae, Planctomycetes, Proteobacteria, Spirochaetes, Tenericutes, Thermotogae and some sequences represent of Euryarcheota: Archaea (Tabla2). The bacterial communities of *D. baritula* were dominated by Proteobacteria (88.20%), Actinobacteria (6.35%), and Firmicutes (4. 35%, Fig.

3.1). In both nectarivorous bird species the rest of bacterial phyla represented less or close to 1% of the total number of sequences (0.46 and 1.00% in *C. latirostris* and *D. baritula* respectively; Table 3.2).

Figure.3.1. Composition per Phyla (%) in the gut of wild birds. At different number of sequences, the left and center side data corresponding at 11274 sequences by each intestinal section for individual, while the right side corresponding the data for each bird (at 52712 sequences). Data obtained with a threshold of 97%.



Act: Actinobacteria, Bac.- Bacteroidetes, Fus.- Fusobacteria, Fir.- Firmicutes, Pro.- Proteobacteria and OP.- Other Groups (include the rest of Phyla that composed the bacterial communities in the gut of *Cynanthus latirostris* (Cyla), *Diglossa baritula* (Diba) and *Passer domesticus* (Pado). Up: upper section, Md: media section and Lw: lower section that includes cloaca and ceca

The bacterial communities in the gut of nectar-feeding birds included 162 families in the case of *C. latirostris* and 152 in *D. baritula*. The number of bacterial genera was higher in *C. latirostris* (616) than in *D. baritula* (557). The most abundant genera (genera with more than 1000 sequences) in *C. latirostris* were: *Acinetobacter, Buttiauxella, Campylobacter, Corynebacterium, Enterobacter, Enterococcus, Pelomonas, Raoultella, Riemerella, Sphingomonas Streptobacillus* and *Yokornell*a. While in the gut of *D. baritula* the most abundant genera were: *Acinetobacter, Aquabacterium, Buttiauxella, Enterobacter, Pelomonas,*

Propionibacterium, Ralstonia, Raoultella and *Sphingomonas*. Finally, the Chao1 indicator showed that the number of bacterial OTUs that we found in the gut of both species was lower than the expected value (10,624.23 ± 70.13 in *C. latirostris*, and 18,223.78 ± 660.86 in *D. baritula*). Indicating that the bacterial diversity in the gut of *C. latirostris* and *D. baritula* could be even higher than the number we reported.

The value of the Shannon Index for *C. latirostris* was 8.02, while in *D. baritula* it showed a value of 7.75. These values indicate that the bacterial communities of the gut of both species had a high bacterial diversity. In both species of birds, gut bacterial communities were constituted by a large number of species with low abundances. Although both nectar-feeding birds differ in the number of genera found in their guts, the bacterial communities of *C. latirostris* and *D. baritula* shared 71.33 % of the genera (similarity index; Table 3.3). However, we found significant differences in abundance of each genus in the bacterial communities of both bird species (Table 3.3).

Changes in bacterial diversity along the gut of the two nectarivorous bird species

The number of sequences and OTUs varied among the three intestinal sections of the gut of our two nectar-eating species. The number of OTUs for each intestinal section was variable for each species. We summarized the information on bacterial diversity for all the different taxonomic levels in Table 3.1. We reported comparisons among the three intestinal sections along the gut for each bird species, and for the same intestinal section between *C. latirostris* and *D. baritula*. Because the number of species that can be found in a sample increases with sample size (Magurran, 2004), and due to the fact that we obtained a different number of sequences in each intestinal section of *C. latirostris* and *D. baritula*, we used a method to balance our sample size in order to conduct appropriate comparisons among the different intestinal sections and between the same portion of the gut for the two bird species. We used the lowest number of sequences found in an intestinal section as our baseline sample size (11,274 sequences from the upper section of *D. baritula*). Although the number of sequences was lower in the medium section of the gut of *C. latirostris*, we decided not to use this sample, since we considered that the low number of sequences we obtained from this section can bias our analysis by making

us lose representability of the real bacterial diversity present in the gut of our two study species. We randomly selected the same number of sequences from each one of the other gut sections of the two bird species. This procedure allowed us to have an identical sequence size, and bypass the bias caused by differences in the sequences number (Magurran, 2004).

Table 3.3. Similarity ($1-\beta_{SIM}$) and Ancova of bacterial dominance for each bacterial community of the gut of different wildbirds. The comparisons conducted a different number of sequences. Data obtained with a threshold of 97%.

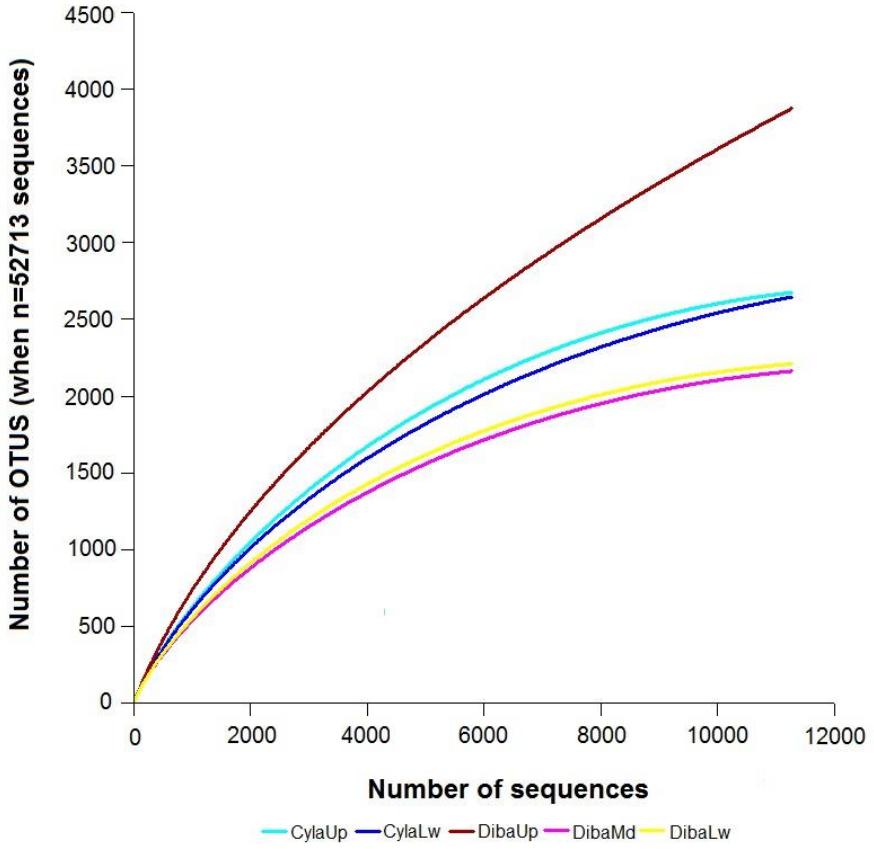
Comparisons	**1-(β-sim)**	**Ancova**
Between individuals		
Cyla vs. *Diba*	0.7133	$F(slope)_{1,1170}$ = 72.1681, P = <0.0001
Between host by intestinal section		
CylaUp vs Cylalw	0.6563	$F(slope)_{1,707}$ = 12.2345, P = 0.0005
Dibaup vs Dibamd	0.6793	$F(slope)_{1,628}$ = 35.3860, P = <0.0001
Dibamd vs Dibalw	0.6504	$F(slope)_{1,559}$ = 16.7108, P = <0.0001
Dibaup vs Dibalw	0.7216	F(slope)1,611 = 3.8675, P = 0.049**7**
CylaUp vs DibaUp	0.6448	$F(slope)_{1,697}$ = 1.2871, P = 0.2570
Cylalw vs Dibalw	0.6960	$F(slope)_{1,621}$ = 41.6145, P = <0.0001
Between species		
Cyla vs Diba		$F(slope)_{1,1161}$ = 52.7118, P = < 0.0001
Cyla vs Pado		$F(slope)_{1,1008}$ = 243.4197, P = < 0.0001
Diba vs Pado		$F(slope)_{1,941}$ = 84.2848, P = < 0.0001

Cyla: *Cynanthus latirostris*, Diba: *Diglossa baritula*, Pado: *Passer domesticus*. Up: upper section, Md: media section and Lw: lower section that includes cloaca and ceca

Bacterial communities found along the gut *Cynanthus latirostris*

When we compared the bacterial diversity of the different sections of the gut, we found that the diversity decreased slightly towards the final portion of the gut (Table 3.1). The comparison of the number of OTUs at the same number of sequences among sections of the gut showed that the upper and the lower sections of the gut were not different in this species (P = 0.05, MacGregor and Payton, 2013; Fig. 3.2), We also found a low difference in the number of bacterial genera present in both the upper (2675 OTUs) and lower section (2646 OTUs, Fig. 3.3 a and c).

Figure 3.2**.** Rarefaction curves for bacterial species richness by intestinal portions of the gut of *C. latirostris* and *D. baritula* at a cut point of 11274 sequences. Data obtained with a threshold of 97%.



Cyla: *Cynanthus latirostris*, Diba: *Diglossa baritula*. Up: upper section, Md: media section and Lw: lower section that includes cloaca and ceca

The RDP´s pipeline indicated that the intestinal bacterial communities in this species were composed mostly by Proteobacteria (62.6 and 77.01% in the upper and lower sections). The most important phyla in the upper section were Fusobacteria, Aquificae, and Actinobacteria, while Firmicutes and Actinobacteria were the most important phyla in the lower section (Fig. 3.1). Together these phyla comprise just over 99% of all bacterial community in the gut of the Broad-billed hummingbird (Fig. 3.1). Bacterial communities were similar in these two portions of the intestine, with the most abundant bacterial genera being present in different numbers along the gut (Fig. 3.3).

When we analyzed bacterial community structure using rank/abundance curves, we found that all intestinal section of the gut had bacterial communities that were dominated by a small number of genera represented by a high number of sequences. While the great majority of the genera were represented only by few sequences (Fig. 3.3a and c). We found that 7 bacterial genera dominated the gut of *C. latirostris* (Fig. 3.3a and c). Bacterial communities of the upper and lower sections were similar in their complexity, diversity, and dominance (Fig. 3.4), suggesting that the gut of *C. latirostris* had a more or less constant bacterial community, even when composition at phyla level can be different, perhaps due to the fact that the diversity of nutrients is similar along the gut of this species.

The most abundant bacterial genera in the gut of *C. latirostris* were *Acinetobacter, Buttiauxella, Campylobacter, Enterobacter, Enterococcus, Sphingobacteria, Streptobacilus* (Fig. 3.3a and c). The Chao1 indicator showed that the number of bacterial OTUS found in both intestinal sections, was slightly below the expected values (Table 3.1), indicating that the bacterial diversity in the gut of *C. latirostris* should be even high than the values reported. While the Shannon Index shows that the upper section contained a higher diversity (7.02), than the lower region (6.90; Table 3.1). These values indicated that the bacterial communities present in the gut of *C. latirostris* had a high diversity (Fig. 3.4).

The comparison of bacterial communities between the two gut sections of this species using a similarity index ($1-\beta_{SIM}$), showed that the bacterial communities of the upper and lower sections of the gut shared 65.63% of bacterial genera (Table 3.3). The β_{SIM} Index showed that bacterial communities gain few bacterial genera along the gut.

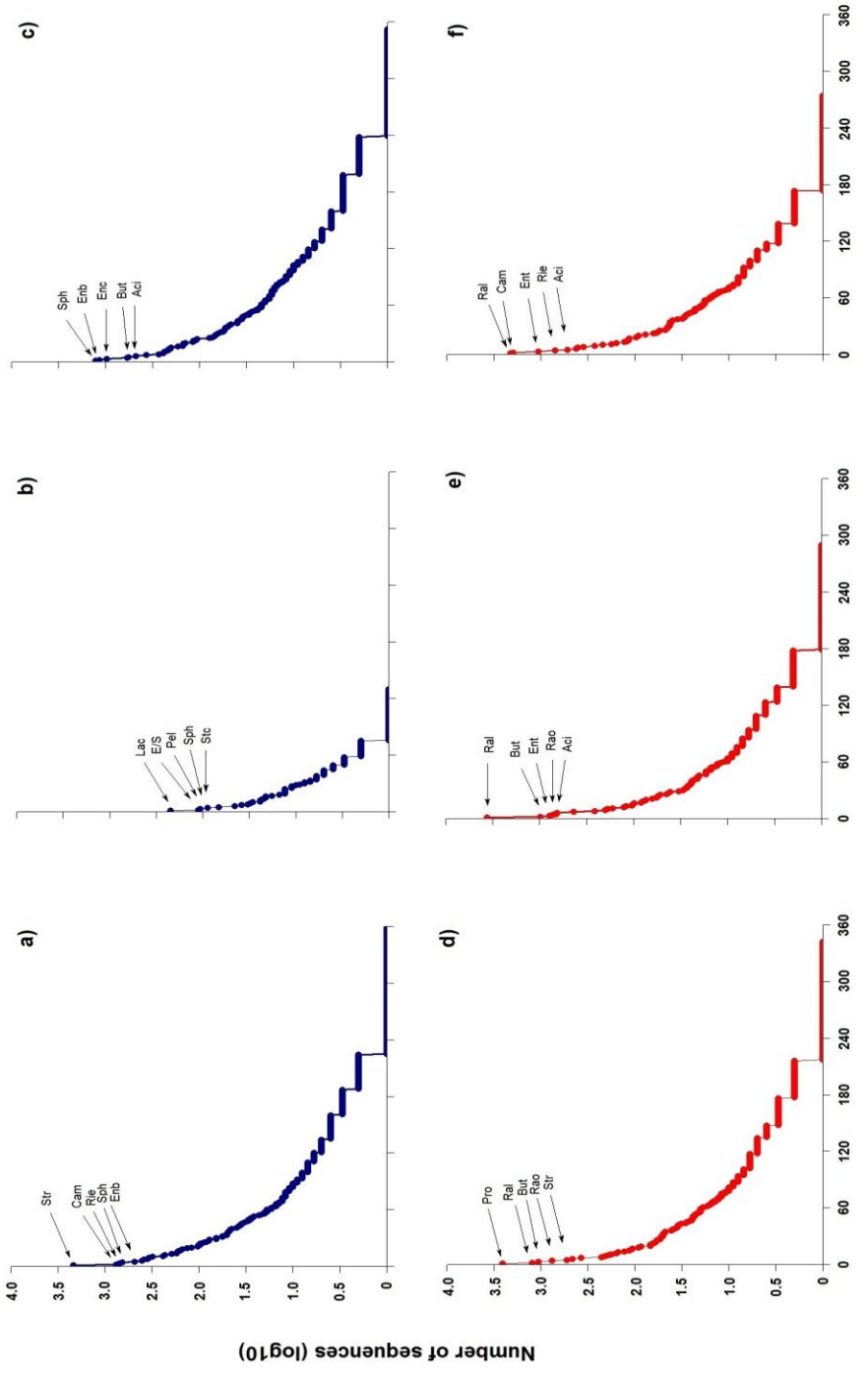


Figure 3.3: Rank-abundance curves of Bacterial Genera present in the intestinal sections of gut of Nectar-feeding birds at 11274 sequences. Data obtained with a threshold of 97%

Aci: *Acidobacteria*, Aqu: *Aquabacterium*, But: *Buttiauxella*, Enb: *Enterobacter*, Enc: *Enterococcus*, Lab: *Lactobacillus*, Par: *Parallactobacillus*, Pel: *Pelomonas*, Ral: *Ralstonia*, Sph: *Sphingomonas*, Stb: *Streptobacillus*, Stc: *Streptococcus*. *Cymanthus latirostris (Cyla)*, *Diglossa baritula* (Diba) a) CylaUp b)CylaMd c) CylaLw e) DibaUp f) DibaMd g) DibaLw

Bacterial communities found along the gut of *Diglossa baritula*

When we compared the bacterial diversity of the three different sections of the gut of *D. baritula*, we found that the diversity decreased towards the final portion of the gut (Table 3.1). The comparison of the number of OTUs at the same number of sequences among sections of the gut showed that the upper, middle and lower sections of the gut were different ($P < 0.05$, MacGregor and Payton, 2013; Fig. 3.2). The number of OTUs was larger in the upper section (3874 OTUs), intermediate in the middle section (2163 OTUs), and lower in the final section (2210 OTUs; Fig. 3.3 d, e and f).

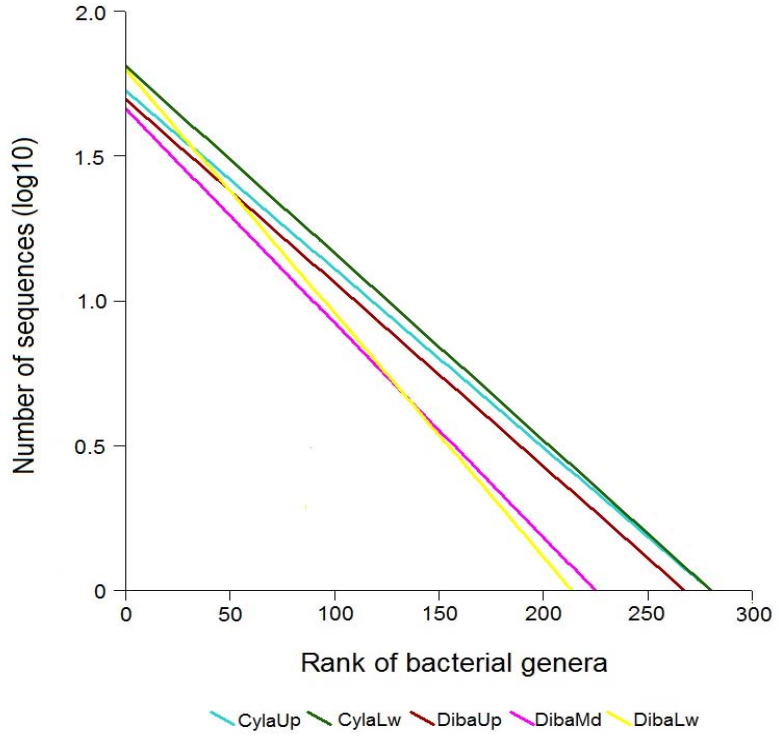
The RDP´s pipeline indicated that the intestine bacterial communities were mostly composed by Proteobacteria (79.4, 95.0 and 85.7% in the upper, middle and lower sections respectively). Following Proteobacteria, the most important phyla were Actinobacteria and Firmicutes. Together these three phyla comprise just over 99% of all the members of the gut bacterial communities (Fig. 3.1). Bacterial communities were similar along the two portions of the intestine of the Cinnamon-Bellied Flowerpiercer; however, the most abundant bacterial genera were present in different numbers in the different sections of the gut (Fig. 3.3).

When we analyzed bacterial community structure using the rank/abundance curves, we found that in each intestinal section of the gut, bacterial communities were dominated by a small number of genera represented by a high number of sequences, while the great majority of the genera were represented by only a few sequences (Fig. 3.3 d, e and f). We found that 9 bacterial genera dominated the gut of *D. baritula*: *Acinetobacter, Buttiauxella, Campylobacter, Enterobacter, Propionibacterium Ralstonia, Raoultella, Riemerella and Sphingobacteria* (Fig. 3.3 a and c). The number of bacterial genera decreased toward the cloaca. Bacterial communities were complex, diverse, and less dominated in the first intestinal section, becoming simpler, less diverse and more dominated by the medium and lower intestinal sections (Fig. 3.4), suggesting that the bacterial diversity in the gut of *D. baritula* depends on the diversity and abundance of nutrients.

The Chao1 indicator showed that the number of bacterial OTUS found in the different intestinal sections, is below the expected values for the upper section, while the middle is lightly below, and the lower portion was well represented (Table 3.1), indicating that the bacterial diversity in the gut of *D. baritula* should be even higher than the values we reported (Table 3.1). The Shannon Index shows that the upper region contained a higher

diversity (7.57), than media (6.55) and the lower region (6.16; Table 3.1). These values indicated that the bacterial communities of the gut of *D. baritula* had a high diversity.

Figure 3.4: Slopes from rank/abundance plots (for both individuals) showing differences in the dominance/evenness of the bacterial communities of the different sections of the gut of the *C. latirostris and D. baritula*. The most diversity and less dominated communities were present in the gut of *C. latirostris*. The structure of bacterial communities can be explained by the availability of food resources in the intestinal ecosystem. Data obtained with a threshold of 97%.



Cyla: *Cynanthus latirostris*, Diba: *Diglossa baritula*. Up: upper section, Md: media section and Lw: lower section that includes cloaca and ceca

The comparison along the gut sections using β_{SIM} showed that the bacterial communities of the upper, media and lower sections shared a high number of bacterial genera,. The first and media section shared 72.13, the media and lower region shared 76.51, while the first and lower section shared 70.96% of the bacterial

genera (Table 3.3). Despite the composition at genera level shows significant differences between contiguous sections (342, 291 and 274 OTUs by the first, media and final section respectively) and between the upper and the lower region (342 and 274; Table 3.3). The similarity index (1- β_{SIM}) showed that bacterial communities gain few bacterial genera along the gut.

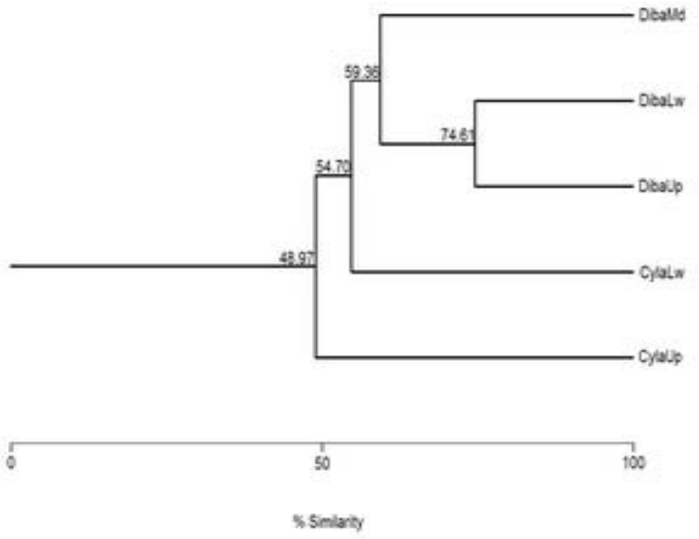
Comparisons between the intestinal bacterial by the gut sections of Nectar-feeding birds

When we compared the bacterial diversity of the different sections of the gut between our two species of Nectar feeding birds, we found that the number of OTUs is major in the upper portion and decreased towards the lower section of the gut. Our data shows the existence of some differences in the number of Phyla, families, genera and OTUs for the same intestinal section of the two nectarivorous birds (Table 3.1),

Our data shows that in both Nectar-Feeding birds, the bacterial communities were dominated by Proteobacteria. These taxa were less abundant in the upper portion of the gut of *C. latirostris* (63.1%) than in *D. baritula* (79.39%; Table 3.2). The proportion of Proteobacteria increased in the lower section of the gut, reaching the 77.45 and 85.75 % of all the sequences for C. latirostris and D. baritula respectively. The proportions of the other Phyla changed in the different sections of the gut of both Nectar-feeding birds without showing a distinct pattern.

Finally we conducted a Bray-Curtis Multivariate Clustering Analysis standardize by the abundance of the sequences to determine the similarity of bacterial communities for the different intestinal section of C. latirostris and D. baritula. This analysis showed that the bacterial communities of the upper and lower section of the gut of C. latirostris have a similarity of 48.97 %, while that the bacterial communities in the gut of D. baritula clustering together, but surprisingly, the upper and the final regions of this species were more similar between them, than with the middle portion of the gut (Fig. 3.5).

Figure 3.5. Bray-Curtis Analysis (for each intestinal section for both individuals) showing clustering of the bacterial communities of the different sections of the gut of the *C. latirostris*, *D. baritula*. Data obtained with a threshold of 97%.



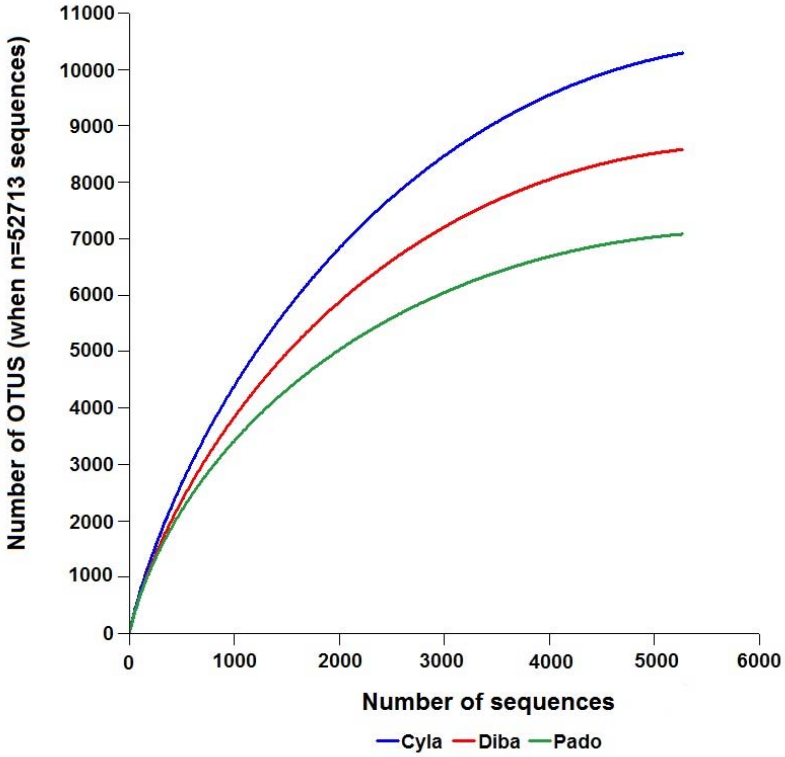
Cyla: *Cynanthus latirostris*, Diba: *Diglossa baritula*. Up: upper section, Md: media section and Lw: lower section that includes cloaca and ceca

Comparison of the gut bacterial communities of two nectarivorous birds with those present in the *Passer domesticus*

For this comparison we analyzed the sequences corresponding to the whole gut for each bird species. The number of sequences we used as a baseline was 52,713. The RDP analysis showed that the number of OTUs was of 10,293 in *C. latirostris*, 8582 in *D. baritula* and 7,081 in *P. domesticus* (Table 3.1). Rarefaction analysis showed that the bacterial species richness for the different species at the same number of sequences was as follows: *C. latirostris* > *D. baritula* > *P. domesticus* 1 (Fig. 3.6). The analysis from the RDP pipeline showed that in the gut of wild birds live between 10 and 17 bacteria Phyla (Table 3.1). The most abundant Phyla were Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria and Proteobacteria. These Phyla represented more than

99% of all the sequences from the bacterial communities. The rest of the Phyla were present in smaller number (≤ 1%, Table 3.2). The composition of bacterial communities for each bird species was different. In *C. latirostris* the bacterial community was dominated by 5 Phyla (Bacteroidetes, Actinobacteria, Fusobacteria, Firmicutes and Proteobacteria), while only three Phyla dominated in the gut of *D. baritula* (Firmicutes, Actinobacteria and Proteobacteria), and the same three, but with a different order of importance, were the dominant ones in the gut of *P. domesticus* (Actinobacteria, Proteobacteria and Firmicutes; Fig. 3.1).

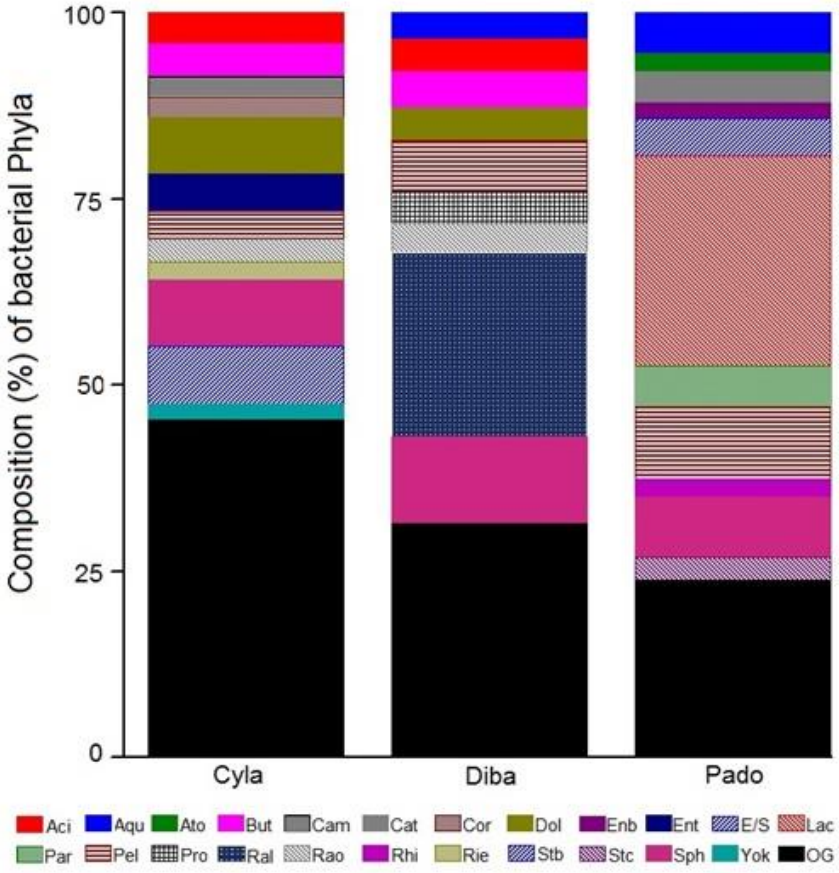
Figure 3.6. Rarefaction curves for bacterial species richness by gut of *C. latirostris*, *D. baritula* and *P. domesticus*, at a cut point of 52713 sequences. Data obtained with a threshold of 97%.



Cynanthus latirostris (Cyla), *Diglossa baritula* (Diba) and *Passer domesticus* (Pado)

We found that the number of Bacterial Families was larger in *C. latirostris*, intermediate in *D. baritula* and lower in *P. domesticus* (Table 3.1). The gut of the birds was dominated by the same bacterial families for three hosts: Enterococcaceae and Lactobacillaceae (Phylum Firmicutes), Burkholderiaceae, Commamonadae, Enterobacteriaceae, Moraxellaceae and Sphingomonadaceae (Phylum Proteobacteria) representing different percentages of the total sequences for each bird (Fig. 3.7). The vast majority of the bacterial families were represented only by a few bacterial sequences (Fig. 3.8).

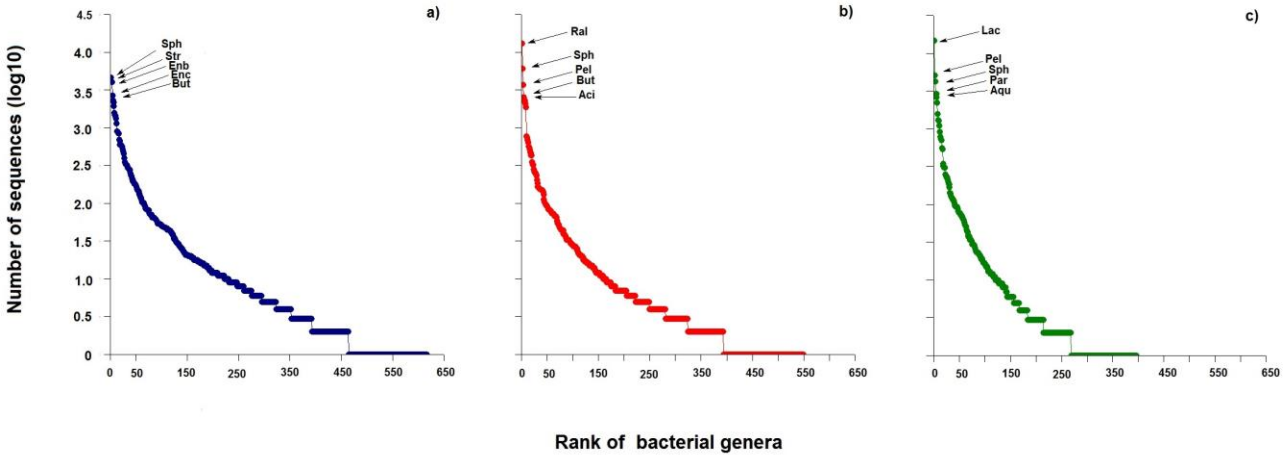
Figure 3.7. Composition per Genera (%) in the gut of wild birds at 52713 sequences. Data obtained with a threshold of 97%.



Aci.- *Acidobacteria*, Aqu.- A*quabacterium*, Ato.- *Atopostipes*, But.- *Buttiauxella*, Cam.- *Campylobacter*, Cat.- *Catellicoccus*, Cor.- *Corynebacterium*, Dol.- *Dolosigranulum*, E/S.- *Escherichia/Shigella*, Enb.- *Enterobacter,* Ent.- *Enterococcus,* Lab.- *Lactobacillus*, Lac.- *Lactococcus*, Par.- *Paralactobacillus*, Pel.- *Pelomonas*, Pro.- *Propioniacerium*, Ral.- *Ralstonia*, Rao.- *Raoultella*,Rie.- *Riemerella*, Sph.- *Sphingomonas*, Stb.- *Streptobacillus,* Stc.- *Streptococcus Yok:Yokernella* and OG.- Other Genera. *Cynanthus latirostris* (Cyla)*, Diglossa baritula* (Diba) and *Passer domesticus* (Pado)

The pipeline's RDP analysis identified 616, 549, and 296 genera of bacteria for *C. latirostris, D. baritula* and *P. domesticus* respectively (Table 3.1; Appendix 2). For nectar-feeding birds the most abundant genera were: *Acinetobacter, Buttiauxella, Enterobacter, Enterococcus, Pelomonas, Ralstonia, Sphingomonas* and *Streptobacillus*. The bacterial genera most abundant in the gut of *P. domesticus* were: *Aquabacterium, Escherichia/Shigella, Lactobacillus, Paralactobacillus, Pelomonas* and *Sphingomonas* (Fig. 3.8).

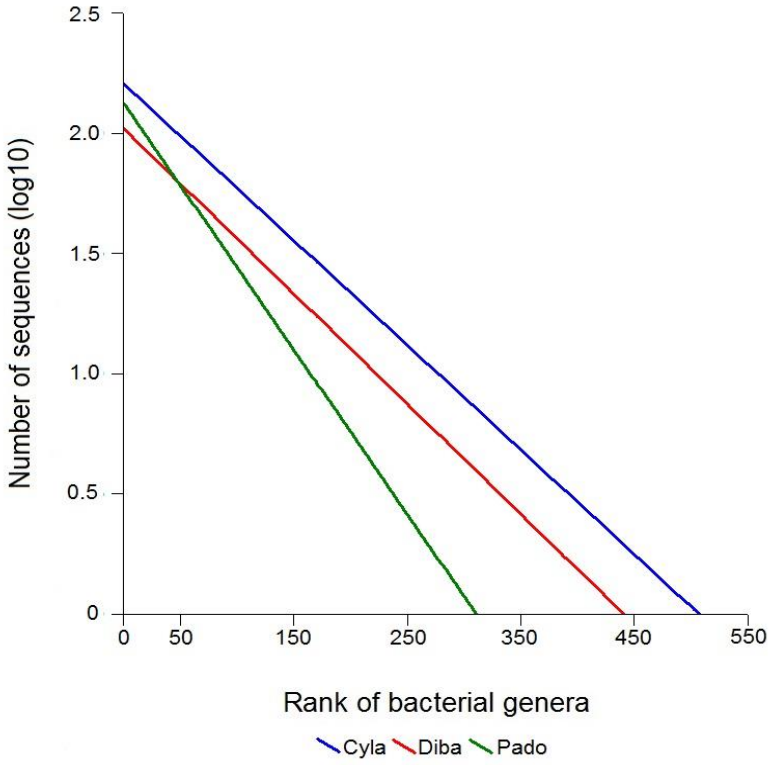
Figure 3.8: Rank-abundance curves of bacterial Genera present in the gut of birds with different diet at 52713 sequences. Data obtained with a threshold of 97%.



Aci: Acidobacteria, Aqu: Aquabacterium, But: Buttiauxella, Enb: Enterobacter, Enc: Enterococcus, Lab: Lactobacillus, Par.- Paralactobacillus, Pel.- Pelomonas, Ral.- Ralstonia, Sph.- Sphingomonas, Stb.- Streptobacillus, Stc.- Streptococcus. Cyla: Cynanthus latirostris, Diba: Diglossa baritula and Pado: Passer domesticus

The Chao1 indicator showed that the number of bacterial OTUs that we found in the gut of each bird species was close to its expected value: 10,624.23 ± 70.13, 87,22.56 ± 42.01 for *C. latirostris* and *D. baritula* respectively, and 7,188.95± 36.99 for *P. domesticus*; Table 3.1). Our analysis shows that the sampled diversity is close to 98 % of the expected diversity. The values of the Shannon Index were 8.02, 7.66 and 7.53 for *C. latirostris, D. baritula, and P. domesticus respectively* (Table 3.1). These values indicate that bacterial communities in the gut of wild birds had a high bacterial diversity regardless of their diet, and that the bacterial communities are constituted by a large number of genera with low abundances (Table 3.1).

Figure 3.9. Slopes from rank/abundance plots (for both individuals) showing differences in the dominance/evenness of the bacterial communities of the different sections of the gut of the *C. latirostris, D. baritula* and *P. domesticus*. The most diversity and less dominated communities were present in the gut of *C. latirostris*. The structure of bacterial communities can be explained by the availability of food resources in the intestinal ecosystem. Data obtained with a threshold of 97%.

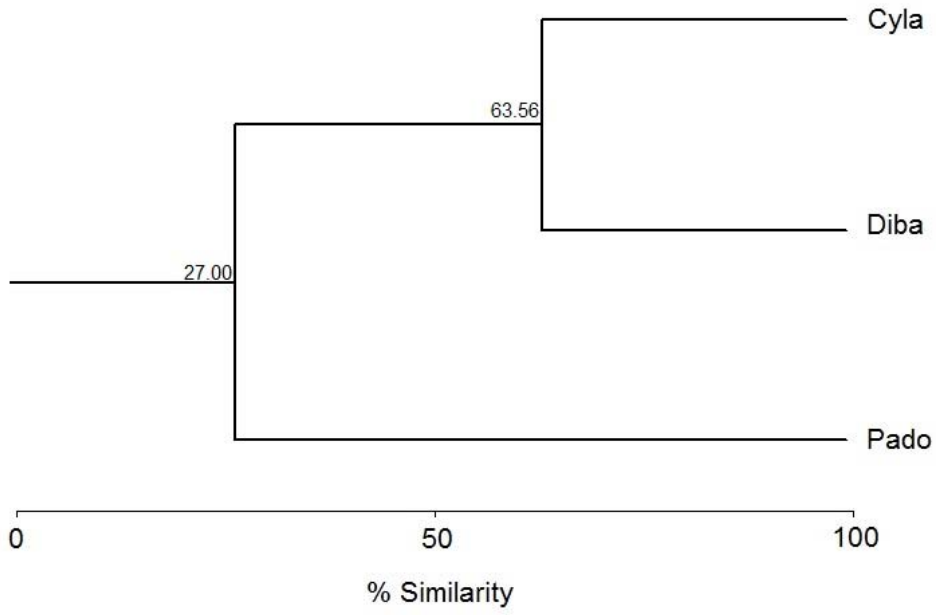


Cynanthus latirostris (Cyla)*, Diglossa baritula* (Diba) and *Passer domesticus* (Pado)

The bacterial communities in the gut of the birds, we found that the composition and number of sequences by genera was highly variable in each host (Fig. 3.8) 616, 549 and 397 for *C. latirostris, D. baritula and P. domesticus*, respectively. Despite the composition by bacterial genera is different for each bacterial communities, the three communities shared a great number of genera (Table 3.3). The comparison of the number of genera at the same number of sequences among bird species showed significant differences among the three bird species (Table 3.3, Fig. 3.7). When we analyzed bacterial community structure at the genus level

using the rank/abundance curves, we found that in each bird, the gut was dominated by a small number of genera represented by a high number of sequences (more than a thousand of sequences), while the great majority of the genera were represented by only a few sequences (Fig. 3.8).

Figure 3.10. Bray-Curtis Analysis showing clustering of the bacterial communities of the different *C. latirostris*, *D. baritula* and *P. domesticus*. Data obtained with a threshold of 97%



Cynanthus latirostris (Cyla), *Diglossa baritula* (Diba) and *Passer domesticus* (Pado)

Bacterial communities were complex, more diverse and less dominated in the two species of nectar-feeding birds than in *P. domesticus* (Fig. 9), suggesting that the presence of large amounts of easily assimilable simple sugars (glucose, fructose, sucrose) allow the existence of diverse bacterial communities. The Bray-Curtis multivariate clustering analysis shows that bacterial communities were more similar between the two species of Nectar-feeding birds, than between *D. baritula* and *P. domesticus*. This analysis shows that the intestinal bacterial communities of the cluster composed by *C. latirostris* and *D. baritula* only had a similarity of 27% with those present in *P. domesticus* (Fig 3.10).

Discussion

Our results show that the gut bacterial communities of nectarivorous birds have higher richness than those found in other species of vertebrates previously studied (mammals, poultry and some wild birds; Amit-Romach *et al.,* 2004; Zoetendal *et al.,* 2006; Wei *et al.,* 2007; Mirón *et al.,* 2014, among others). We obtained between 52713 and 53996 sequences for all the gut of C. latirostris and D. baritula, although is hard compared our results with other studies due that the type of sample used, ADN extraction methods, the size of sequences, the number of sequences and the analysis realized, have notable differences. From our two study species, the most diverse gut bacterial community was found in the gut *C. latirostris* (10293) and the less diverse was present in *D. baritula* (9621). Surprisingly our data shows that the gut bacterial communities of nectarivorous birds were more complex and diverse, that those previously found in wild birds and mammals with more complex diets (Salanitro *et al*., 1978; Pacheco *et al*., 2004; Scupham *et al*., 2008; Benskin *et al*., 2010; Godoy-Vittorino *et al*., 2010, 2012; Liu *et al*., 2011; Mirón *et al.,* 2014). Our data shows that at the same number of sequences, the number of OTUs found in the gut of *P. domesticus*, an omnivorous bird (7082 OTUs), was significantly lower than the number of OTUs found in nectar-feeding birds (Table 3.1). Our data also show notable differences in the composition of Phyla, genera, and in the dominance per genera among the bacterial communities present in the gut of the studied birds. Although the intestinal bacterial communities of the three species shared a large number of bacteria genera, the Bray-Curtis analysis indicated that the two nectarivorous birds had very similar intestinal bacterial communities, regardless of the phylogenetic relationships between them. Our results suggest that diet is a more important factor in the conformation of bacterial communities in birds than phylogeny. We also found that the bacterial community composition at the same number of sequences (11274 sequences) differed among gut sections, in both birds; the number of OTUs is greater in the upper region and decreases toward the lower portion of the gut (Table 3.1). We believe that this is consequence of changes in the nutrient diversity and abundance, and the different habitat conditions present along the gut (Stevens and Hume, 1998; Klasing 2005)

Why nectarivorous birds have more complex intestinal bacterial communities than animals with other diets?

Similarly to our results, previous studies that compared the intestinal bacterial communities of mammals with different diets, found that species feeding from plants, including nectarivorous species, had more complex bacterial communities than those present in the gut of species with nutritional more complex diets (carnivorous, herbivorous, omnivorous; Ley *et al*., 2008; Phillips *et al*., 2012). Nectar is the simpler food that exists in the world (Nicolson and Fleming, 2014). It consists basically on a sugar solution (sucrose, glucose and fructose), with very small amounts of amino acids and minerals (Baker, 1978; Martinez del Rio, 1990; Baker *et al*., 1998; Tsahar *et al.,* 2006; Nicholson and Thornburg, 2007). This makes nectar a highly energetic food with low protein and lipid content (Nicolson and Fleming, 2014). Due the hummingbirds need to maintain high metabolic rates and the need to maintain flight, nectar feeding birds, need to digest nectar meals and assimilate the sugar present extremely rapidly (Nicolson and Fleming, 2014). As a result, the guts of nectarivorous birds tend to present high concentrations of carbohydrates (Karasov and Cork, 1994). On one hand, carbohydrates are an excellent substrate for a large number of bacterial species to grow, on the other hand, it is possible that the microbiome of these birds are producing y exchange for the carbohydrates essential amino acids, lipids and vitamins in the gut of Hummingbirds and Flowerpiercer. Curiously in other ecological systems (terrestrial ecosystems), the diversity of species inside natural communities tend to be correlated not with the amount of resources available, but with the diversity of such resources (Magurran, 2004, Cadott *et al*., 2011). As a result, ecosystems with high availability of one resource tend to present communities that are poor in species and extremely dominated (Magurran, 2004). Moreover, what has been discussed above, we cannot ignore the fact that the nectar is a large reservoir of bacteria and fungi (Aizenberg-Gershtein *et al*., 2013; Engel *et al.,* 2013; Good *et al*., 2013).

Hummingbirds and Flowerpiercers display remarkably high rates of sugar assimilation (higher than 99%; Lopez-Calera *et al*., 1997; McWhorter and Martinez del Rio, 2000; Schondube and Martinez del Río, 2004). Mathematical models using sucrose hydrolysis data indicate that both of the nectarivorous study species are able to assimilate all the sugar they ingest, or even larger amounts than those they normally ingest (McWhorter and Martinez del Rio, 2000; Schondube and Martinez del Río, 2004). This implies that the bacterial communities

present in the gut of hummingbirds and Flowerpiercer could be competing for the sugars present in the diet of their hosts. However, since the diet of nectarivorous animals lacks other crucial nutrients (like Nitrogen, vitamins among others; Baker, 1978; Martinez del Rio, 1990; Baker *et al*., 1998; Tsahar *et al.,* 2006; Nicholson and Thornburg, 2007), gut bacterial communities of these birds could be acting as important symbionts to their hosts, by using carbohydrates as a substrate to grow, synthesizing other nutrients, and helping with the recycling of nutrients that could be used by their hosts.

A larger number of animal populations are limited not by the availability of energy, but by the scarcity of nitrogen (Bosque and Pacheco, 2000; McWhorter and Martinez del Rio, 2000; Tsahar *et al.,* 2006). Nectar and fruit pulp contain very low levels of protein, and as a result, animals that feed on these diets must have low nitrogen requirements (Tsahar *et al.,* 2005; Tsahar *et al.,* 2006; Nicholson and Thornburg, 2007; Cronck and Ojeda, 2008). However, even with low nitrogen requirements, nectar by itself cannot cover the nitrogen requirements of most nectarivorous animals (Nicholson and Fleming, 2003; Tsahar *et al*., 2006; Nicholson and Thornburg 2007). Some authors suggested two complementary mechanisms through which nectar-feeding birds can meet their nitrogen requirements: they can eat some insects (Stiles, 1995; Schondube and Martinez del Rio, 2003; Cronk and Ojeda 2008), or in they can recycled urinary nitrogen, by moving uric acid microspheres from the cloaca to the intestine using a reflux mechanism (Campbell and Braun, 1989; Braun 1999; Braun 2003). In the intestine, bacteria are essential for such recycling converting uric acid to other compounds that the birds could assimilate (Karasawa and Maeda, 1994; Preest *et al.,* 2003). Preest *et al.,* (2003) has reported the presence of bacteria with uricase activity in the gastrointestinal tract of the Anna Hummingbird (*Calypte anna*; Preest *et al.,* 2003), and Tsahar *et al.,* (2005) found post-renal urine modification in the Yellow-vented Bulbul that could be the result of bacterial degradation. These observations suggest the existence of bacterial nitrogen recycling processes associated to bacteria in the final section of the gut of nectarivorous and frugivorous birds. However the contribution of bacteria to the nitrogen balance of nectarivorous birds remains to be demonstrated (Preest *et al*., 2003; Tsahar *et al*., 2005; Davila *et al*., 2013).

The *composition of bacterial communities in the gut of nectarivorous birds*

Not surprisingly, our results show that the intestinal bacterial communities of *C. latirostris* and *D. baritula* are different from those reported for mammals, and similar to those found in other bird species. In the vast majority of mammals the bacterial communities are dominated by Firmicutes and Bacteroidetes (Eckburg *et al.,* 2005; Ley *et al.,* 2008; McKenna *et al.,* 2008). But in the case of birds, intestinal bacterial communities can be dominated by Firmicutes and other phyla like Actinobacteria (Banks *et al.,* 2009; Benskin *et al.,* 2010), Bacteroidetes (Scupham *et al.,* 2008) and Proteobacteria in wild and captive Parrots (Xenoulis *et al.,* 2010), Hoatzins (Godoy-Vittorino *et al.,* 2010; 2012), House Sparrows (Miron *et al.,* 2014) and Zebra Finches (Benskin *et al.,* 2010). Our data shows that the bacterial communities in the gut of *C. latirostris* and *D. baritula* were dominated by Proteobacteria (70.28% and 88.19% for *C. latirostris* and *D. baritula* respectively), and a low percentage of Actinobacteria and Firmicutes. So, our results were similar but slightly different from previous studies.

The predominance of Proteobacteria is evident along of gut of *C. latirostris* and *D. baritula,* increasing towards the final portion of their guts (Table 3.2). However, the distribution the other bacterial Phyla (Actinobacteria and Firmicutes) did not followed a clear pattern. Our data is insufficient to draw conclusions about the distribution of the different bacterial Phyla in the intestines of nectarivorous birds, and it is necessary to sample more individuals of our study species, and to increase the number of nectarivorous birds sampled to determine this issue. In other bird species (like the House sparrow; (Mirón *et al.,* 2014) we can assume that changes in the composition of bacterial Phyla along the gut can be associated with changes in the function of the gut, like digestion, absorption, and waste management (Randall *et al.,* 2002; Klasing, 2005; Scupham *et al.,* 2008).

Functional role of bacteria in the gut of nectarivorous birds

Both functionally and taxonomically, bacteria are one of the most diverse groups of organisms in our planet (Horner-Devine *et al.,* 2004; Buckley, 2004; Rajilić-Stojanović *et al.,* 2007). To classify bacteria functionally is difficult because they have the ability to metabolize a wide spectrum of substrates, and have the capacity to change their metabolism depending on the substrates present on their environment (Lu *et al.,* 2003b; Horner-Devine *et al.,* 2004; Zoetendal *et al*., 2006; Rajilić-Stojanović *et al*., 2007; Lu and Santo Domingo, 2008). The most important group of bacteria in the gut of *C. latirostris* and *D. baritula* was the Proteobacteria. The Proteobacteria are a major phylum of bacteria, but the role of the Proteobacteria in the gut of birds is unknown and remains to be explored (Kersters*, et al*., 2006). Several authors have explored the functional diversity of bacteria within the gut of honey bee (*Apes miellifera),* and found that some γ-Proteobacteria species have genes encoding pectin-degrading enzymes likely involved in the breakdown of pollen walls and with the transport of carbohydrates (Engel *et al.,* 2012).

The second more abundant bacterial phylum in the gut of *C. latirostris* and *D. baritula* was Actinobacteria. Actinobacteria were one of microorganisms producing the most antibiotics, some drugs (Allen *et al*., 2010; Jiang *et al*., 2013; Chen *et al*., 2014), bioactive metabolism, enzyme inhibitors and enzymes (Jiang *et al.,* 2013) and masters of decomposition of complex nutrients, their presence in birds with this simple diet is interesting since ants use Actinobacteria to maintain their fungal “gardens” (Good *et al*., 2013). The results of recent studies shows that the nectar is not sterile, it will find large amounts of Actinobacteria, the presence or absence of specific bacteria can alter the chemical characteristics of the nectar and the quality and healthy of the nectar (Aizenberg-Gershtein *et al*., 2013; Good *et al*., 2013). It is possible that nectarivorous birds could use them also to maintain their sugar loaded gut healthy.

On the other hand, nectarivorous birds obtain all their energy from floral nectar, they ingest insects to obtain protein, or to survive when nectar is scarce in the environment (Stiles, 1995; Schondube and Martínez del Rio, 2003; Cronk and Ojeda 2008). The presence of this bacterial group could be related to their small, but constant, ingestion of insects.

Firmicutes were also abundant in the gut of our study species. This bacterial Phylum is considered a normal and abundant component of gut bacterial communities in a large number of vertebrates (Zoetendal *et al.,* 1998; Ley *et al*., 2008). Firmicutes are also capable to metabolize complex compounds of the diet, and recently have been related with obesity in human and mice (Bäckhed *et al*., 2004; Turnbaugh and Gordon, 2009). They seem to be related to fat and carbohydrate metabolism, and they increase their numbers when their host ingests high caloric diets (Hildebrand *et al*., 2009; Turnbaugh and Gordon, 2009; Zhao *et al*., 2013; Tilg and Kaser, 2014). Additionally, bacterial communities with large numbers of Firmicutes were able to extract more calories from the diet for their hosts (Bäckhed *et al*., 2004; Hildebrand *et al*., 2009; Turnbaugh and Gordon, 2009). While this could be related with obesity in some animals, it could benefit nectarivorous birds like hummingbirds that present extremely high metabolic rates (McWhorter and Martínez del Rio, 2000; Tsahar *et al*., 2005). Even if the bacterial diversity in the gut of *C. latirostris, D. baritula* and *P. domesticus* is enormous, this may not necessarily indicate they are functionally unimportant as symbionts. Is also important to considerer that the hummingbirds, necessitating fast transit times (time for food to pass through the gut; Nicolson and Fleming, 2014), that can be a possibility that a great number of bacteria to be "hitchhiking" (Ley *et al*., 2006; Banks *et al.,* 2009)

The role of phylogeny and diet in determining the diversity and composition of intestinal bacterial communities in birds

The role that phylogeny and diet play in conforming intestinal bacterial communities of vertebrates has been explored in recent years by several authors (Nelson *et al.,* 2003; Sundset *et al.,* 2004, 2007; Ley *et al.,* 2008; Phillips *et al.,* 2012). Our study provides strong evidence that diet is the most important factor determining the composition of bacterial communities in the gut of birds. Our study species are considered nectarivorous birds, these species belong to two families that have evolved nectarivory independently: Trochilidae (*Cynanthus latirostris*), and Emberizidae (*Diglossa baritula*; Cronk and Ojeda, 2008; Schondube *et al.,* 2003). From a Phylogenetic perspective, hummingbirds and Flowerpiercer are very distant, with *D. baritula* being much closer to Passer *domesticus* (family Passeridae) than to *C. latirostris* (Ericson and Johansson, 2003). Our analysis showed that the bacterial communities of the guts of the two species of nectarivorous birds clusters together,

while the bacterial communities of *P. domesticus* formed a different group.

The similarity of the bacterial communities present in the guts of *C. latirostris* and *D. baritula* suggests that diet is the most important factor that influences the conformation of bacterial communities in the gut of birds. *C. latirostris* and *D. baritula* can be found in the same localities feeding on the same flowers, however, the individuals we studied were captured in different sites (more than 400 km away). For this reason we can discard a geographic and a feeding effect on the similarity in the composition of bacterial communities in the gut of these two nectarivorous birds. This suggests that bacterial communities in these two birds are responding to similar pressures generated by the simplicity and homogeneity of their diets.

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Discusión general

Los datos obtenidos en el presente trabajo, muestran una diversidad bacteriana inesperada en el intestino de las aves de estudio, no solo en el número de OTUs (entre 4436 y 10293), sino en su composición, ya que las comunidades bacterianas están conformadas por miembros de entre 10 y 18 Phyla bacterianos. La comunidad bacteriana más diversa se encuentra en *C. latirostris*, intermedia en *D. baritula* y menos diversa en *P. domesticus*. En conjunto, las aves de estudio tienen comunidades más complejas que las reportadas previamente en la literatura (Godoy-Vitorino *et al.,* 2008; Scupham *et al.,* 2008; Kong *et al.,* 2010). Dado que existen diversos métodos para obtener la diversidad bacteriana es difícil comparar los resultados de nuestro trabajo con otros, debido a que: a) las secuencias bacterianas se han obtenido usando diferentes técnicas (amplificación del gen 16S, ARISA, librería de genes(Lu *et al.* 2003, Gong *et al.,* 2007, Scupham *et al.,* 2008; Banks *et al.,* 2009), b) el tipo de tejido analizado (Lucas y Heeb, 2005; Banks *et al.,* 2009; Ruìz-Rodriguez *et al*., 2009; White *et al.* 2010) c) el método de extracción de ADN utilizado (kits vs. métodos tradicionales, Norgard *et al*., 2005, Mogan *et al.,* 2010), d) dieta de las especies estudiadas (Godoy-Vitorino *et al.,* 2008; Scupham *et al.,* 2008, Banks *et al.,* 2010) y e) el número de secuencias obtenido por cada muestra (ya que muchos trabajos solo reportan algunos cientos de muestras).

Existen diversas razones por las cuales la diversidad bacteriana en las comunidades bacterianas en el intestino de las aves de estudio es más grande en comparación con los resultados obtenidos por otros autores. Comenzando por el procesamiento de las muestras previo a la extracción de DNA. El método de análisis de las muestras utilizadas en el presente trabajo es, desde, muchos puntos de vista diferente a otros trabajos. Nosotros usamos todo el tejido intestinal, incluyendo su contenido. El procesamiento de las muestras, antes de la extracción del DNA, nos permitió determinar la presencia y distribución de las bacterias que componen cada uno de los hábitats del ecosistema intestinal; las bacterias que forman biopelículas, las que se encuentran en la superficie de la pared intestinal, la mucosa intestinal, las que encuentran sobre las partículas de alimento, las que flotan libremente en el ambiente intestinal y en el caso de *P. domesticus*, las que se encuentran en excretas (Miron *et al*., 2001; Rabiu y Gibson, 2002; Akada *et al*., 2003; Manco *et al.,* 2010). El método que elegimos para la extracción del DNA bacteriano, permite la separación de las células eucariotas de las bacterianas, mientras

que el proceso de lisis física y química asegura el procesamiento de un mayor número de células bacterianas de muestras intestinales (Norgard *et al*., 2005). El conjunto de condiciones descritas anteriormente influencian de manera importante no solo el número de secuencias obtenidas en nuestro análisis sino también en el número de OTUs reportadas para cada sección intestinal (y por tanto de todo el intestino).

El estudio de las comunidades bacterianas en el intestino de aves silvestres está en sus albores, los resultados de estos trabajos muestran que las comunidades bacterianas en el intestino están compuestas de al menos 20 Phyla bacterianos (Eckburg *et al*., 2005, Zoetendal *et al.,* 2006, Ley *et al*., 2008). Dichas comunidades están dominadas por miembros de los Phyla Firmicutes y Bacteroidetes (Lu *et al*., 2003, Gong *et al*., 2008, Scupham *et al*., 2008). En algunas aves como gallinas y pavos, este patrón es similar (Zhu *et al*., 2002, Lu y Santo Domingo 2008, Scupham *et al*., 2008). Sin embargo en otras aves, los grupos dominantes son Actinobacteria (Banks *et al*., 2008), Proteobacteria (Xenoulis *et al*., 2010).

Los resultados del presente trabajo muestran que las comunidades bacterianas del intestino de *C. latirostris, D. baritula* y *P. domesticus* están dominadas por Proteobacteria, y en menor proporción por Firmicutes y/o Actinobacteria. Los resultados de nuestro análisis no permiten establecer un patrón definido en cuanto a la riqueza de especies bacterianas a lo largo del intestino, aunque los datos sugieren que la diversidad bacteriana disminuye hacia la parte final del intestino. Esta disminución de la diversidad bacteriana podría ser debida a las diferencias funcionales existentes en el ecosistema intestinal. De acuerdo con esto, la sección superior del intestino (la región más cercana al estómago) es la zona que tiene mayor diversidad y riqueza bacteriana, debido a que en esta zona existe una mayor disponibilidad de recursos (es la zona de digestión; Stevens y Hume, 1998, Randall *et al.,* 2002). La zona intermedia del intestino tiene una diversidad bacteriana intermedia, al ser una zona donde se termina de metabolizar los nutrientes que no han sido digeridos y comienzan a ser degradados algunos compuestos. Finalmente la zona final de la región intestinal presenta menor diversidad y riqueza, debido a que en esta región la disminución de los recursos es notable (Stevens y Hume, 1998, Randall *et al.,* 2002). La porción final del intestino incluye la cloaca, en la que confluyen los conductos finales del tracto digestivo, el aparato urinario y el reproductor (Stevens y Hume 1998, Randall *et al.,* 2002). Por lo tanto, las bacterias presentes en la cloaca pueden tener diferentes orígenes y no ser propiamente parte del ecosistema intestinal (Phillips y Heeb 2003, Ruiz-Rodríguez *et al*., 2009).

Las bacterias son uno de los grupos funcionalmente más diversos desde todos los puntos de vista: taxonómico, genético y metabólico, y tienen una gran habilidad para metabolizar diversas sustancias dependiendo de los recursos disponibles en el ambiente (Buckley, 2004), lo que hace difícil definir la funcionalidad de los diferentes grupos bacterianos presentes en diversos ecosistemas. Se piensa que a pesar de que la diversidad bacteriana taxonómica puede es enorme, la diversidad funcional puede ser baja (debido a que los sustratos presentes en el ecosistema intestinal son limitados) y existe un alto grado de especialización y redundancia funcional (Zoetendal *et al.,* 2006, Turroni *et al.,* 2008, Serikov *et al.,* 2010). El papel funcional de las bacterias en el ecosistema intestinal está íntimamente relacionado con la composición de la dieta del hospedero. Así pues, bajo esta premisa, y dependiendo de la dieta del hospedero, se podría esperar encontrar bacterias con actividad proteolítica, recicladoras de nitrógeno (Karasawa y Maeda, 1994, Prees *et al.,* 2003), queratolíticas y bacterias capaces de degradar diversos polisacáridos vegetales.

Dado que el néctar es el alimento más sencillo de la tierra, compuesto básicamente de tres azucares en diversas proporciones, con pequeñas cantidades de aminoácidos, vitaminas y minerales (Martínez del Río 1990, Schondube *et al.,* 2004), es de fácil digestión y los azucares que lo componen son de asimilación pasiva (Schondube *et al*., 2003), una comunidad bacteriana intestinal compleja no sería útil desde el punto de vista funcional. Sin embargo los resultados del presente trabajo muestran que las aves nectarívoras tienen comunidades complejas, con un alto número de especies bacterianas en el ecosistema intestinal ¿Cómo explicar esta aparente contradicción?

La explicación más sencilla seria el exceso de carbohidratos en el intestino *C. latirostris* y *D. baritula*, dado que un gran número de bacterias pueden metabolizar diversos carbohidratos (Lovanh *et al*., 2007, Walter *et al*., 2008), éstas podrían establecerse en el intestino de aves nectarívoras, porque existe disponibilidad de recursos, pero existe la posibilidad de que no le brinden un beneficio directo al hospedero. Mientras que la dieta de las aves granívoras (como en el caso de *Passer domesticus*) es rica en almidón, polisacárido abundante en las semillas (Pacheco *et al.,* 2008) y los lactobacilos degradan el almidón en maltosa, maltotriosa y glucosa (Stevens y Hume, 1998). Quizá la dominancia de Firmicutes en *Passer domesticus* esté relacionada directamente con la digestión de los carbohidratos complejos presentes en su dieta.

Por lo que el siguiente paso en el estudio de las comunidades bacterianas que habitan el intestino de *C.*

latirostris, D. baritula y *P. domesticus*, es establecer el papel funcional que tienen las bacterias en el ecosistema intestinal, haciendo diversos estudios, tanto en laboratorio (cultivos celulares, para determinar uso de fuentes de carbono y nitrógeno), como utilizando diversas herramientas moleculares (proteomica, transcriptomica).

Es necesario realizar más estudios para determinar la diversidad real de bacterias en el ecosistema intestinal y para establecer el papel qus tienen en el ecosistema intestinal. El siguiente paso, es comparar las comunidades bacterianas de un gran número de especies de aves nectarívoras, pertenecientes a diferentes familias. Los resultados obtenidos en este trabajo son insuficientes para establecer si la dieta es el factor más importante en la conformación de las comunidades bacterianas, por lo que es necesario hacer una comparación a mayor escala.

Conclusiones

• La diversidad de bacterias presentes en el intestino de *Cynanthus latirostris, Diglossa baritula y Passer domesticus* es mayor a lo reportado para otras aves. Esta gran diversidad podría explicarse debido a:

a) el tipo de muestra utilizada permitió obtener el DNA de las bacterias que habitan los distintos nichos intestinales a lo largo de todo el intestino.

b) el método de obtención del DNA.

c) la disponibilidad de sustratos derivados de la dieta del hospedero (en el caso de *Passer domesticus*).

d) la disponibilidad de una gran cantidad de carbohidratos (en el caso de ambas aves nectarívoras).

• Las comunidades bacterianas en el intestino de las aves de estudio, están compuestos por al menos 18 Phyla bacterianos, el intestino del gorrión doméstico (*Passer domesticus*) está dominado por Firmicutes y Proteobacteria (en diversas proporciones a lo largo del intestino). Mientras que el intestino de las aves nectarívoras (*Cynanthus latirostris* y *Diglossa baritula*) está dominado por Proteobacteria, Firmicutes y Actinobacteria. Las diferencias en la composición a nivel de Phyla de las comunidades bacterianas pueden estar relacionadas directamente con la dieta de los hospederos. En el caso de los mamíferos y algunas aves de corral, la presencia de Bacteroidetes y Firmicutes podría estar asociada al metabolismo de carbohidratos complejos (como la celulosa). Mientras que la dominancia en el tracto digestivo de las aves de estudio es predominantemente Proteobacteria y esto podría estar asociado al metabolismo de carbohidratos simples.

• Los resultados presentados en este trabajo son insuficientes para describir un patrón sobre la distribución y diversidad de las comunidades bacterianas en el intestino de las aves en general. Sin embargo, los datos parecen indicar que la diversidad bacteriana disminuye hacia la porción final del intestino, dependiendo de la cantidad y calidad de los nutrientes disponibles en el ambiente intestinal.

• Los datos de *C. latirostris* y *D. baritula*, muestran que la composición de las comunidades bacterianas de estos hospederos son más similares entre sí que con las comunidades encontradas en el intestino de *Passer domesticus*. Los resultados sugieren que la dieta tiene un papel importante en la conformación de las comunidades bacterianas.

• El estudio pueden sentar antecedentes para la investigación de las comunidades bacterianas en el tracto gastrointestinal de aves nectarívoras con diferente origen filogenético.

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Appendix 1: Genera found in each of the intestinal sections of the House Sparrow

Genus	PD1			PD2		
	UP	MD	LW	UP	MD	LW
Abiotrophia	32	34	7	19	48	
Acetanaerobacterium					184	
Acetobacter	1					
Acetohalobium				2		
Acetonema				7	1	
Achromobacter			1	3	4	
Acidomonas	2			1	2	
Acidovorax	115	1	1			
Acinetobacter	729	50	10	31	3	
Actibacter	1					
Aeromonas	250					
Aestuariimicrobium	73	4	1	21	4	
Afipia	3					
Agaricicola		1				
Agromonas	35			2	2	
Albidiferax	3	1				
Algicola	1					
Alicycliphilus	15			1		
Alkalibacterium	23	57	3	6	5	
Alkanindiges	62	15		2	3	
Allobacillus	1					
Allofustis	1					
Allomonas	3					
Aminobacter	9	1	1	4	3	
Amorphus				1		
Anaerobacter	31	1		2	1	
Anaerococcus		1		6		
Anaerospora				1		
Anaerovibrio				3		
Aquabacterium	3140	153	51	813	249	4
Aquamicrobium		2		2		1
Aquincola	3			1		
Aquisalibacillus	1					
Aquitalea	1					
Arenicella					1	
Arsenophonus	1					
Arthrobacter	2			1		

Appendix 1: Genera found in each of the intestinal sections of the House Sparrow (continuation)

Genus	PD1			PD2		
	UP	MD	LW	UP	MD	LW
Asaia					1	
Aspromonas		1		1		
Asticcacaulis	8			1		
Atopobacter	1	2		5		
Atopostipes	776	608	169	176	167	1
Auraticoccus		1		2		
Auritidibacter	2					
Azohydromonas	1	1				
Azomonas	1					
Azorhizobium	1			3	2	
Azorhizophilus	4	1		1		
Azovibrio	3			1	1	2
Balnearium				1		
Balneatrix	1				1	
Balneimonas				1		
Bangiophyceae		1		5		
Bavariicoccus	11	7		6	4	
Beggiatoa	2					
Bergeriella	16			2	1	
Bermanella	2					
Biostraticola	10	2				
Blastobacter	18	1		5	3	
Blastomonas	217	20	2	68	31	1
Blastopirellula				4		
Bosea	1					
Brachymonas	13					
Bradyrhizobium	42	1		2	1	
Brenneria	3					
Brevibacterium			1			
Brevundimonas	39	1	1	2	4	
Brochothrix					1	
Brooklawnia	4					
Brucella	31	2		16	4	
Burkholderia		1				
Buttiauxella	201	1				4
Caedibacter		2		3		

Appendix 1: Genera found in each of the intestinal sections of the House Sparrow (continuation)

Genus	PD1			PD2		
	UP	MD	LW	UP	MD	LW
Caldimonas	3					
Camelimonas				1		
Campylobacter		1	6			
Catellicoccus	58	974	16	1516	827	
Catelliglobosispora	25	3		3	1	
Caulobacter	120	2	1	9	4	
Cedecea	13					
Cellulosilyticum	25				127	
Cerasibacillus	1					
Chelatococcus	2			1		
Chitiniphilus			1			
Chloroherpeton				1		
Chryseobacterium	2	2				
Chryseoglobus	6	1		1		
Citrobacter	91	40	44	1	1	
Clavibacter				3		
Clavibacter	10	2				
Cloacibacterium	95					
Clostridium sensu stricto	21					
Clostridium XlVb	1					
Coenonia	2					
Comamonas	262	20	8	2		
Conexibacter	1					
Corynebacterium	4	1		2		
Cosenzaea	33	19	1			
Croceicoccus	1				2	
Cronobacter	7	3				
Curvibacter	38			7		
Cycloclasticus	1					
Cystobacteraceae				1		
Daeguia				1		
Deinococcus				1		
Delftia	23	4	1	39	8	
Derxia	4	1				
Desemzia	1	1	1		1	
Desulfatiferula				3		
Desulfoglaeba		1				

Appendix 1: Genera found in each of the intestinal sections of the House Sparrow (continuation)

Genus	PD1			PD2		
	UP	MD	LW	UP	MD	LW
Desulfovermiculus				1		
Devriesea				1		
Diaphorobacter	25					
Dickeya	2					
Dolosigranulum	577	332	39	434	278	1
Duganella	106					1
Ectothiorhodosinus	1					
Edaphobacter					3	
Elioraea				1		
Elizabethkingia	14					
Empedobacter	30			1	1	
Endozoicomonas	6					
Enterobacter	142	1			4	15
Enterococcus	210	29	25	4	6	
Epilithonimonas	16					
Erwinia	157			1		
Erythromicrobium	62	35		36	35	
Escherichia/Shigella	2160	1180	632	3	4	
Euzebya				2		
Exiguobacterium	491					
Falsibacillus	1					
Ferribacterium	2					
Ferrimonas	1					
Fervidicella				1		
Filibacter	1					
Filimonas				2		
Filomicrobium				1		
Flavihumibacter					1	
Flavisolibacter				1	2	
Flavitalea					1	
Flavobacterium	10	2	2	8	1	
Fodinibacter					1	
Fontibacillus		1				
Frigoribacterium	20	4		6	4	
Frondihabitans	2					
Fulvimarina	2			1		
Galbibacter				1		

Appendix 1: Genera found in each of the intestinal sections of the House Sparrow (continuation)

Genus	PD1			PD2		
	UP	MD	LW	UP	MD	LW
Gemella	1					
Geothermobacter		1		4		
Gibbsiella	61	5				
Giesbergeria	3					
Gluconacetobacter	1				1	
Gp1				2		
Gp3				1		
Gp4				1		
Granulibacter					1	
Granulicatella	5					
Granulicella	3					
Gulosibacter	4			1		
Hafnia	40				1	
Hahella	1					
Haloactinobacterium	4					
Haloplasma	1					
Halotalea	1					
Herbaspirillum	1					
Herbiconiux	37			9	2	
Herminiimonas	4					
Hoeflea	4		1			
Holdemania					1	
Hydrogenoanaerobacterium			1			
Hydrogenovibrio	1					
Hydrotalea		2			1	
Hylemonella	11			3	1	
Hyphomicrobium				2		
Iamia				21		
Ideonella					1	
Ilumatobacter				11		
Inhella	2					
Iodobacter	3					
Isobaculum	217	122	140	40	5	1
Janthinobacterium	12				1	
Jeongeupia	1					
Jeotgalicoccus	1					
Jishengella				3		

Appendix 1: Genera found in each of the intestinal sections of the House Sparrow (continuation)

Genus	PD1			PD2		
	UP	MD	LW	UP	MD	LW
Kineosphaera	4			4		
Kinneretia	82	10		12	9	
Kistimonas	5					
Klebsiella	98	2		1		
Klugiella	1			2		
Kluyvera	228					
Kocuria	3					
Kozakia					3	
Labedella	4	1		1	1	
Labrys		1				
Lacticigenium	1					
Lactobacillus	11614	3572	2377	1925	2893	1
Lactococcus	48	8	4		109	914
Lactovum	11	4		1	458	15
Lampropedia	138	11	2		3	
Lawsonia	1					
Leclercia	114	41	3	1	6	20
Leifsonia	251	8	4	60	7	
Leminorella	4					
Leptobacterium					1	
Leptothrix	32			2	1	
Limnobacter	2	2			1	
Limnohabitans	16					
Lucibacterium	1					
Lysobacter				1		
Macrococcus	1					
Macromonas	7	1		1	2	
Malikia	3				1	
Mangrovibacter	206	141	19	3	9	
Marinilactibacillus	43	7	1		3	
Marisediminicola	5			2		
Massilia	130	1				
Meganema				1		
Melissococcus	82	120	4	49	111	15
Mesoflavibacter		1		4		
Mesorhizobium				1		
Metascardovia	1					

Appendix 1: Genera found in each of the intestinal sections of the House Sparrow (continuation)

Genus	PD1			PD2		
	UP	MD	LW	UP	MD	LW
Methylibium	37	4		12	3	
Methylobacterium	9			1		
Methylocaldum				1		
Methylocapsa		1				
Methylocella	1					
Methylonatrum	3					
Methylophilus	2	1				
Methylorhabdus	1					
Methylosphaera	1					
Methylotenera	14			8	1	
Methyloversatilis			1	1		
Methylovirgula				1		
Microbacterium	29	5	1	10		
Micrococcus				3		
Microterricola	32	4		6	8	
Microvirga				3		
Microvirgula	1					
Millisia		2				
Mitsuaria	297	60	7	62	51	4
Modicisalibacter	8					
Mycetocola	6			1	1	
Mycobacterium	3			2		
Mycoplana	11	2		2		
Natronocella			1			
Naxibacter	5		1	2		1
Neptuniibacter	1	2				
Nesterenkonia	15	1				
Nevskia				2		
Niabella		1		1		
Nitratireductor	2			4	4	
Nitriliruptor	1			1		
Nitrincola	1					
Nitrobacter	17			3		
Nitrosospira	1				1	
Nosocomiicoccus				1		
Novosphingobium	2		1	1	1	
Nubsella				1		

Appendix 1: Genera found in each of the intestinal sections of the House Sparrow (continuation)

Genus	PD1			PD2		
	UP	MD	LW	UP	MD	LW
Obesumbacterium	60	3	1	2		
Ochrobactrum	4	1		2	2	
Oerskovia				2		
Okibacterium	2					
Oligotropha						
Oligotropha	20			1		
Orbus	1	1				
Oribacterium				1		
Orientia	1	1		1	7	
Ottowia	4			1	1	
Oxalicibacterium	1					
Oxalobacter	10					
Oxalophagus	3			1		
Paenibacillus					2	
Paenisporosarcina	7					
Paenochrobactrum	85	18		16	19	2
Pannonibacter	3					
Pantoea	56				50	98
Paracoccus	7					
Paraferrimonas	2					
Paralactobacillus	539	774	1337	1640	85	
Paramoritella	11			1		
Parapusillimonas				1	1	
Parvimonas				1		
Pasteuria				3		
Paucibacter	2			2		
Pectobacterium	5					
Pedobacter				2		
Pelomonas	5243	371	116	1679	354	1
Perlucidibaca	20	1			1	
Petrobacter	1					
Phascolarctobacterium					5	
Phenylobacterium	15	1				
Phocoenobacter	2	1				
Phycicola	6			1	1	
Phyllobacterium	33	2		10	8	1
Pilibacter	212	103	13	1	3	1

Appendix 1: Genera found in each of the intestinal sections of the House Sparrow (continuation)

Genus	PD1			PD2		
	UP	MD	LW	UP	MD	LW
Pisciglobus	9	3		1		
Piscirickettsia	1					
Planococcaceae_incertae_sedis	1					
Planomicrobium		2				
Plantibacter	1					
Plesiomonas	1				3	
Polynucleobacter				4		
Pontibaca			1			
Ponticaulis	1					
Porphyrobacter	24	3		7	4	
Pragia	4					
Propionibacterium	857	17	27	268	10	5
Proteiniborus					1	
Proteiniclasticum	2					
Proteiniphilum				1	1	
Proteus	13	2				
Pseudaminobacter	14	3		3		
Pseudochrobactrum	41	8		15	11	
Pseudoclavibacter	10	1		4	3	
Pseudofulvimonas				1		
Pseudolabrys	1					
Pseudomonas	10	1				
Pseudoramibacter	1					
Pseudorhodoferax	104	2		13		
Pseudosphingobacterium	1	1				
Pseudoxanthobacter	8	2		3	4	
Psychrosphaera	2					
Pullulanibacillus	1					
Quadrisphaera				14		
Quatrionicoccus	2					
Rahnella	11	3				
Ralstonia	9	2	8	7	1	
Raoultella	414	2		1	1	28
Reinekea	6					
Rheinheimera	3					
Rhizobacter	1499	58	23	379	2	
Rhizobium	3			1		

Appendix 1: Genera found in each of the intestinal sections of the House Sparrow (continuation)

Genus	PD1			PD2		
	UP	MD	LW	UP	MD	LW
Rhodoblastus	1	2		2	1	
Rhodococcus				1		
Rhodoferax	5	2				
Rhodopila	2					
Rhodopirellula				6		
Rhodopseudomonas	1			1	1	
Riemerella	1		1			
Rivibacter	62	11	1	15	12	1
Roseibaca	1					
Rothia	3				1	
Rubrivivax	31	2		4	6	
Rugamonas	91			2		
Ruminobacter	3	2				
Rummeliibacillus	10					
Saccharibacillus	17					
Saccharococcus		1				
Saccharofermentans		1		4		
Salana	1			1		
Salinarimonas	6	1				
Salinibacterium	18			2	1	
Salinicoccus	11		1			
Salinimonas	6	1	2	1		
Salirhabdus	1		1			
Salmonella	40	17	26			1
Samsonia	20	3				
Sandaracinobacter	97	16		11	10	
Sandarakinorhabdus	3	3		4	6	
Sarcina	2					
Schlesneria				5		
Schumannella	2	3			2	
Schwartzia	1					
Sediminicola				1		
Sediminimonas				1		
Serinibacter				1		
Serpens	2					
Serratia	134			6	1	
Shimazuella	1					

Appendix 1: Genera found in each of the intestinal sections of the House Sparrow (continuation)

Genus	PD1			PD2		
	UP	MD	LW	UP	MD	LW
Shimwellia	21				5	
Shinella	4			1	2	
Simiduia	5					
Simonsiella		1		2		
Simplicispira	17			2	1	
Singulisphaera		3				
Sinorhizobium	42	3		7	12	
Sinosporangium				1		
Sodalis	13	1				3
Solibacillus					1	
Solimonas	1					
Sphingobacterium		1				
Sphingobium	7	2			1	
Sphingomonas	4056	381	144	1630	226	2
Sphingopyxis	389	74	6	165	166	4
Sphingosinicella	1019	67	7	129	139	
Stakelama	687	97	6	104	136	1
Staphylococcus	66	2	705	15	1	
Starkeya	1			1		
Stenotrophomonas	2	18	7			
Steroidobacter	4			1		
Streptococcus	1302	410		15	10	1
Streptophyta NC	5			4		
Succinimonas	1					
Sulfuritalea	1			2		
Tatumella	32					
Telluria	6					
Telmatospirillum	2			3		
Tepidamorphus	1	3	1			
Tepidicella					1	
Tepidimonas			1			
Terriglobus				2		
Tessaracoccus				1	1	
Thermodesulfobium	1					
Thermomicrobium				2		
Thermotalea				1		
Thermus				1		

Appendix 1: Genera found in each of the intestinal sections of the House Sparrow (continuation)

Genus	PD1			PD2		
	UP	MD	LW	UP	MD	LW
Thiobacter	2		1			
Thioclava	3					
Thiophaeococcus	2					
Thiorhodospira	1					
Thorsellia	1					
Tistrella	1					
Tolumonas	1					
Trabulsiella	16	3				
Trichococcus	32					
Tropheryma				4		
Truepera	1					
Turicibacter					2	
Turicibacter	15					
Umboniibacter	1					
Vagococcus		1				
Variovorax	27					
Vasilyevaea	1					
Verminephrobacter	4					
Vitreoscilla	87	9		12	14	
Vogesella	17					
Wautersia		1				
Wautersiella	40		1			
Weissella	8	5				
Wohlfahrtiimonas	1					
Wolinella						1
Xanthobacter	2					
Xenophilus	2					
Xylanimicrobium	2				2	
Xylophilus	5			1		
Yersinia	1					
Yimella	2					
Yokenella	194	66	12	3	9	1
Zimmermannella	2					
Zobellella	2					
Zunongwangia	1					
Zymomonas	18			3	5	
Total of Sequences	43048	10377	6042	11897	7103	1152

Appendix 2 Bacterial Genera found in the gut of three wild birds: *Cynanthus latirostris* (Cyla), *Diglossa baritula* (Diba) and *Passer domesticus* (Pado)

Género	Cyla	Diba	Pado
Abiotrophia	86	12	93
Acaricomes	20	2	
Acetanaerobacterium	3		113
Acetivibrio	2		
Acetohalobium		1	
Acetonema		1	5
Achromobacter	53	1	5
Acidimicrobium	1		
Acidisphaera	2		
Acidomonas	2	5	2
Acidothermus		1	
Acidovorax	65	32	77
Acinetobacter	2200	2315	558
Actinaurispora	1		
Actinomycetospora	4		
Actinotalea	1		
Advenella	3	1	
Aeribacillus		1	
Aeriscardovia		2	
Aerococcus	5		
Aeromonas	18	31	169
Aestuariimicrobium	48	152	65
Afipia	3	3	2
Agaricicola	12	2	1
Agarivorans	1	4	
Aggregatibacter	84	2	
Agreia	1	2	
Agrococcus		1	
Agromonas	23	33	23
Albibacter	9		
Albidiferax	19	84	4
Albidovulum	4	6	
Alcanivorax	6	11	
Alicycliphilus	17	7	12
Alicyclobacillaceae_incertae_sedis	2		
Alicyclobacillus	4		
Alishewanella	2	1	

Género	Cyla	Diba	Pado
Alkalibacterium	12	26	70
Alkaliflexus		1	
Alkalimonas		1	
Alkanibacter		1	
Alkanindiges	232	253	54
Allisonella	1		
Alloactinosynnema	4		
Allobacillus			1
Allobaculum		2	
Allocatelliglobosispora	1		
Allofustis	1	1	1
Allokutzneria	7		
Allomonas	4	2	2
Alloscardovia	3		
Altererythrobacter	4	3	
Alysiella		1	
Amaricoccus	7	17	
Aminobacter	53	74	15
Amycolicicoccus	49	3	
Anaerobacillus		1	
Anaerobacter	1	1	23
Anaerococcus	20	45	6
Anaeroglobus		1	
Anaeromyxobacter			3
Anaerosphaera	2	3	
Anaerospora		1	1
Anaerovibrio	5		3
Anaplasma	9	1	
Angustibacter	1		
Aquabacterium	841	1861	2892
Aquamicrobium	2	1	5
Aquaspirillum	1	11	
Aquimonas	1	3	
Aquincola	1	1	
Aquisalibacillus			1
Aquitalea			1
Arcobacter	8	7	

Appendix 2. Bacterial Genera found in the gut of three wild birds (continuation)

Género	Cyla	Diba	Pado
Arenicella	1		
Arenimonas	2	7	
Arsenophonus	14	1	1
Arthrobacter	9		3
Asaia		1	1
Aspromonas	18	3	2
Asticcacaulis	45	7	6
Atopobacter	5	1	2
Atopobium	102	1	
Atopococcus		1	
Atopostipes	45	98	1280
Aurantimonas		1	
Auraticoccus	7	4	3
Auritidibacter	2	12	
Avibacterium	327	7	
Azoarcus		1	
Azohydromonas	2	1	1
Azomonas	19	5	1
Azonexus		1	
Azorhizobium	7	15	5
Azorhizophilus	12	3	4
Azospirillum	2		
Azotobacter		3	
Azovibrio	5	5	2
Bacillariophyta		1	
Bacillus	43	10	1
Bacteroides	5		
Balnearium		1	
Balneatrix	12	14	2
Balneimonas	5	3	1
Bangiophyceae		22	5
Bavariicoccus	21	14	19
Bdellovibrio	1		
Beggiatoa	1		
Beijerinckia	13	83	
Bellilinea	1		
Belnapia	2		
Bergeriella	34	27	10
Bergeyella	81		
Bermanella	54	45	2
Beutenbergia	2		
Bibersteinia	71	3	
Bifidobacterium	1		
Biostraticola	18	21	9
Blastobacter	41	58	21
Blastomonas	597	746	214
Blastopirellula			1
Bosea	16	1	1
Brachymonas	9	21	9
Brachyspira	1	2	
Bradyrhizobium	37	74	27
Branchiibius	1	2	
Brenneria	29	1	1
Breoghania	5	9	
Brevibacterium	2		1
Brevundimonas	280	44	34
Brochothrix	101	15	
Brooklawnia	2	6	3
Brucella	11	14	38
Bryobacter		2	
Budvicia	45	2	
Burkholderia			1
Buttiauxella	2283	2528	143
Caedibacter			2
Caenimonas	2	1	1
Caldimonas	1	3	3
Camelimonas	1		1
Caminicella	1		
Campylobacter	1464	9	1
Carnimonas	4	1	
Caryophanon	1		
Catellibacterium	4		
Catellicoccus	206	148	2235
Catelliglobosispora	62	68	22
Catenibacterium	1		
Caulobacter	173	74	78
Cedecea	54	33	8

Appendix 2. Bacterial Genera found in the gut of three wild birds (continuation)

Género	Cyla	Diba	Pado
Celeribacter	19		
Celerinatantimonas	10	5	
Cellulomonas		20	
Cellulosilyticum	323	4	95
Cellvibrio	5		
Centipeda	1		
Cerasibacillus		1	
Cesiribacter		2	
Cetobacterium	4		
Chelativorans		2	
Chelatococcus	2	27	3
Chelonobacter	6		
Chitinimonas		1	
Chitiniphilus	3	6	
Chlorarachniophyceae	4	1	
Chlorophyta	2	12	
Chromatium		2	
Chryseobacterium	34		2
Chryseoglobus	2	3	3
Citreicella	21	1	
Citrobacter	153	81	117
Clavibacter	3	15	12
Cloacibacterium	21	5	58
Clostridium III	3		
Clostridium IV	1		
Clostridium sensu stricto			12
Clostridium XI		1	
Clostridium XIX	17	1	
Clostridium XlVb			1
Clostridium XVIII		1	
Coenonia	6		2
Comamonas	597	435	201
Conchiformibius		2	
Conexibacter			1
Corynebacterium	1442	75	4
Cosenzaea	15	22	33
Coxiella		1	
Crabtreella	2	3	
Croceicoccus	7	7	2
Crocinitomix		1	
Cronobacter	5	3	5
Cryptanaerobacter	1	1	
Cucumibacter	6	1	
Cupriavidus		12	
Curtobacterium	9		
Curvibacter	8	53	34
Cycloclasticus	1		1
Daeguia	5	3	1
Defluviicoccus	1	3	
Dehalogenimonas	1		
Deinococcus			1
Delftia	484	150	51
Demequina	5		
Denitratisoma	2	1	
Dermatophilus	1		
Derxia	7	7	4
Desemzia	7	3	2
Desulfatiferula	5	2	2
Desulfatirhabdium	6	10	
Desulfitispora	3		
Desulfocella		2	
Desulfonatronospira	1	1	
Desulfonauticus	1		
Desulforegula		1	
Desulfovermiculus			1
Devosia	48	3	
Devriesea	7	6	1
Diaphorobacter	7	5	17
Dickeya	1		2
Dinoroseobacter	2		
Dolosigranulum	31	95	1098
Dongia	4	1	
Duganella	7	4	73
Dyadobacter		7	
Ectothiorhodosinus	1	1	1
Edaphobacter			2
Edwardsiella		1	

Appendix 2. Bacterial Genera found in the gut of three wild birds (continuation)

Género	Cyla	Diba	Pado
Elioraea			1
Elizabethkingia	4		11
Empedobacter		3	22
Endozoicomonas	16	4	3
Enhydrobacter	65	4	
Ensifer	16	18	
Enterobacter	4026	2305	116
Enterococcus	2673	80	184
Epilithonimonas	57	1	7
Erwinia	145	89	96
Erysipelothrix		3	
Erythrobacter	4	4	
Erythromicrobium	127	65	128
Escherichia/Shigella	297	331	2645
Eudoraea		1	
Euglenida		1	
Euzebya			2
Exiguobacterium	1	7	307
Exilispira	17	12	
Facklamia		1	
Faecalibacterium		1	
Falsibacillus	9		1
Fangia	2		
Ferribacterium	5	3	1
Ferrimonas	1		
Ferrithrix	11	11	
Ferroplasma	1	3	
Ferruginibacter	3		
Fervidicella			1
Fibrisoma	82	1	
Filibacter	1		1
Filimonas	21	33	1
Filomicrobium	2		1
Finegoldia		5	
Flavihumibacter		1	1
Flavisolibacter	1	2	3
Flavitalea			1
Flavobacterium	15	2	13
Flectobacillus		4	
Fontibacillus	1		1
Formivibrio	4	9	
Frateuria	5		
Frigoribacterium	8	12	23
Frondihabitans	2		
Fulvimarina	16	6	2
Fulvimonas	1	1	
Fulvivirga		4	
Fusibacter	1		
Gaetbulibacter	1		
Galbibacter	1		1
Gallaecimonas		4	
Gallibacterium	10		
Gelidibacter		1	
Gemella		5	1
Gemmata		6	
Gemmobacter	2		
Geopsychrobacter		1	2
Georgenia	7		
Georgfuchsia		1	
Geothermobacter	1	2	
Gibbsiella	93	27	44
Giesbergeria			3
Gilvimarinus	1		
Gluconacetobacter			2
Goodfellowiella	11	2	
Gordonia	1	2	
Gordonibacter	6		
Gp1			1
Gp3	4	11	
Gp4		15	2
Gp6	8		
GpI	1	69	
GpIIb	1		
GpIV		19	
GpIX		2	
Granulibacter			1
Granulicatella	2	8	4

Appendix 2. Bacterial Genera found in the gut of three wild birds (continuation)

Género	Cyla	Diba	Pado
Granulicella			3
Guggenheimella		1	
Gulosibacter	5	1	4
Haematobacter	14	3	
Haemophilus		2	
Hafnia	213	151	25
Hahella	5	3	
Haliscomenobacter	9		
Haloactinobacterium	30	18	1
Halobaculum		1	
Haloechinothrix	2		
Halolamina		1	
Halonotius		3	
Halopelagius	2		
Haloplasma	1		1
Halorhodospira		2	
Halotalea	3	1	1
Halothiobacillus	6		
Hamadaea		3	
Hansschlegelia	2		
Helcobacillus	1	2	
Helicobacter	45		
Heliophilum		1	
Hellea	5		
Herbaspirillum	1		1
Herbiconiux	8	7	30
Herminiimonas	3		1
Hippea	1		
Hoeflea	181	185	4
Holdemania			1
Howardella	2	2	
Hoyosella	1		
Humibacter	1	1	
Humihabitans		3	
Hydrocarboniphaga		2	
Hydrogenimonas	10	1	
Hydrogenophaga		7	
Hydrogenophilus	4		
Hydrogenothermus	3		
Hydrogenovibrio	11		1
Hydrotalea			2
Hylemonella	71	155	10
Hyphomicrobium			2
Hyunsoonleella		1	
Iamia		2	15
Ideonella	19	44	1
Ilumatobacter	1		5
Inhella	2	2	1
Intrasporangium	15		
Iodobacter	4	5	1
Isobaculum	65	27	345
Isochromatium		7	
Isoptericola	1		
Jannaschia	5		
Janthinobacterium		1	10
Jeongeupia		2	1
Jeotgalicoccus	3		
Jhaorihella	1		
Kerstersia		1	
Kineosphaera	12	18	6
Kingella	1		
Kinneretia	47	37	74
Kistimonas	32	28	2
Klebsiella	525	690	69
Klugiella	1	1	1
Kluyvera	84	67	140
Kocuria	71	49	3
Kushneria		1	
Labedella			6
Labrys			1
Lachnobacterium	3		
Lacticigenium	1		1
Lactobacillus	334	775	14821
Lactococcus	22	72	727
Lactovum	8	6	321
Lamprocystis	3	16	

Appendix 2. Bacterial Genera found in the gut of three wild birds (continuation)

Género	Cyla	Diba	Pado
Lampropedia	190	163	105
Laribacter	2	7	
Lawsonia			1
Leadbetterella		1	
Lebetimonas		1	
Lechevalieria	1		
Leclercia	862	284	123
Legionella	1	1	
Leifsonia	199	517	230
Leminorella	1	3	1
Lentzea	3		
Leptobacterium	3		1
Leptothrix	41	92	24
Leucobacter		2	1
Leuconostoc	72	106	
Limnobacter	10	39	4
Limnohabitans	20	13	13
Listeria		21	
Listonella	2		
Lonepinella	1		
Longispora	2		
Lucibacterium	1		1
Luteimicrobium		1	
Luteimonas	2	5	
Luteipulveratus	9	13	
Macrococcus			1
Macromonas	16	5	9
Malikia	3	5	2
Mangrovibacter	59	54	237
Mannheimia	20	5	
Marihabitans	12	1	
Marinactinospora		1	
Marinicella	8		
Marinilactibacillus	2	8	34
Marinitoga		1	
Marinobacter	1		
Mariprofundus	1		
Marisediminicola	26	11	3
Marispirillum	2		
Marivita	3		
Martelella	1		
Massilia	34	17	93
Meganema	2	1	1
Melissococcus	103	29	252
Meridianimaribacter	3		1
Mesoflavibacter		1	1
Mesorhizobium	17		1
Metascardovia			1
Methanomicrobium		1	
Methanopyrus	2		
Methermicoccus	8		
Methylarcula	1	4	
Methylibium	11	12	38
Methylobacillus		8	
Methylobacterium	2	132	6
Methylocaldum	1		1
Methylocapsa	2	1	
Methylocella	3	2	
Methylococcaceae			1
Methylococcus	1	9	
Methylohalomonas	1		
Methylonatrum			1
Methylophilus			1
Methylopila	9	9	
Methylorhabdus	3		1
Methylosoma	1	3	
Methylosphaera	6	3	1
Methylotenera	7	9	13
Methylothermus	4	1	
Methyloversatilis			1
Methylovirgula	8	9	1
Methylovulum		1	
Microbacterium	17	5	29
Micrococcus	10	2	2
Micropruina	4		
Microscilla		2	
Microterricola	63	20	30

Appendix 2. Bacterial Genera found in the gut of three wild birds (continuation)

Género	Cyla	Diba	Pado
Microvirga	8	1	3
Microvirgula	22	38	
Millisia	294	2	2
Miniimonas	2		
Mitsuaria	151	235	314
Modicisalibacter	31	16	5
Moraxella	6		
Morganella	2	4	
Mucilaginibacter		1	
Mucispirillum		1	
Mycetocola	8	10	4
Mycobacterium		5	3
Mycoplana	19	23	9
Mycoplasma	23	28	
Myroides		2	
Nakamurella	1		
Nannocystaceae		3	
Natronobacillus	1	1	
Natronocella		1	1
Naxibacter			5
Neochlamydia		1	
Neptuniibacter	13	6	1
Nesiotobacter		1	
Nesterenkonia	1		10
Nevskia			2
Niabella		5	2
Nisaea	2	5	
Nitratifractor	7	1	
Nitratireductor	18	47	6
Nitratiruptor	1		
Nitriliruptor		1	2
Nitrincola	6		
Nitrobacter	12	4	13
Nitrococcus	2	1	
Nitrosococcus	3	7	
Nitrosospira	1	78	2
Nosocomiicoccus	4	4	1
Novosphingobium	39	59	4
Nubsella			1
Obesumbacterium	892	431	39
Oceanibaculum	1	2	
Oceanicola	1		
Oceaniserpentilla	14	2	
Oceanisphaera	3		
Ochrobactrum	14	30	7
Oenococcus		1	
Oerskovia		4	2
Okibacterium	2		2
Oleispira		1	
Oligotropha	11	9	15
Olsenella	7		
Orbus	73	6	2
Orientia	6	5	6
Ornithinibacillus		2	
Oscillibacter	3		
Ottowia	1	3	4
Oxalicibacterium			1
Oxalobacter	1	6	7
Oxalophagus	1		2
Oxobacter		1	
Paenibacillus			1
Paenisporosarcina			3
Paenochrobactrum	61	46	95
Pandoraea	1	1	
Pannonibacter	4	8	3
Pantoea	15	33	124
Parachlamydia	1		
Paracoccus	135	17	6
Paracraurococcus		1	
Paraferrimonas		3	2
Paralactobacillus	83	30	2922
Paramoritella	3		8
Paraoerskovia	8	1	
Parapusillimonas	5		1
Parascardovia	2		
Parasegetibacter		1	
Parvibaculum	2	1	

Appendix 2. Bacterial Genera found in the gut of three wild birds (continuation)

Género	Cyla	Diba	Pado
Parvimonas		2	
Parvularcula	2		
Pasteuria	12		2
Paucibacter	1	1	3
Paucimonas	2	3	
Paucisalibacillus		4	
Pectobacterium	44	15	4
Pedobacter			2
Pedomicrobium	2		
Pelagibaca	2	2	
Pelagicola	11	35	
Pelistega	1	4	
Pelomonas	1944	3716	5107
Pelosinus		2	
Peptoniphilus		8	
Peredibacter	9	2	
Perexilibacter	3		
Perlucidibaca	67	25	14
Persicivirga	5		
Petrimonas		1	
Petrobacter	11	17	1
Phascolarctobacterium			3
Phaselicystis	2		
Phenylobacterium	10	8	11
Phocaeicola	1		
Phocoenobacter	456	14	1
Photobacterium		1	
Phycicola	64	4	6
Phycisphaera		1	
Phyllobacterium	394	254	38
Pilibacter	21	29	230
Piscicoccus	6		
Pisciglobus	1		8
Piscinibacter		3	
Piscirickettsia	1		
Planifilum	2		
Planobacterium	28		
Planococcaceae_incertae_sedis			1
Planomicrobium		1	
Planosporangium		1	
Plantactinospora		1	
Plantibacter	2		
Pleomorphomonas	9	7	
Plesiomonas	324	15	3
Polynucleobacter		1	3
Pontibaca	15	9	
Ponticaulis	10		1
Porphyrobacter	54	33	29
Porticoccus	2	2	
Pragia	20	3	1
Promicromonospora		3	
Propionibacterium	903	2190	788
Propionicicella	4	1	
Propionicimonas		1	
Propioniferax	2	2	
Propionimicrobium	1	2	
Propionivibrio	1	2	
Prosthecochloris		1	
Proteiniborus			1
Proteiniclasticum	1	2	
Proteus	1	4	9
Pseudacidovorax			1
Pseudaminobacter	119	67	15
Pseudochrobactrum	28	24	46
Pseudoclavibacter		3	16
Pseudoflavonifractor		1	
Pseudofulvimonas		1	
Pseudogulbenkiania	3		
Pseudolabrys	6	1	1
Pseudomonas	280	101	9
Pseudonocardia	1		
Pseudorhodoferax	39	44	81
Pseudosphingobacterium	2	1	2
Pseudospirillum	1		
Pseudoxanthobacter	5	3	12
Pseudoxanthomonas	11	6	
Psychrilyobacter	251	7	

Appendix 2. Bacterial Genera found in the gut of three wild birds (continuation)

Género	Cyla	Diba	Pado
Psychrobacillus	1	1	
Psychrosinus	1	2	
Psychrosphaera	10	1	2
Pullulanibacillus	1		1
Quadrisphaera	3		9
Quatrionicoccus	4	4	2
Rahnella	15	4	11
Ralstonia	580	13074	17
Ramlibacter	1		
Raoultella	1589	2072	307
Reinekea		2	2
Renibacterium	4		
Rheinheimera	2		3
Rhizobacter	178	467	1303
Rhizobium	16	39	4
Rhodoblastus	1		4
Rhodococcus	6	1	1
Rhodocyclus		2	
Rhodoferax	3	4	3
Rhodoglobus		1	
Rhodomicrobium	3	5	
Rhodopila			1
Rhodopirellula			5
Rhodopseudomonas	2	2	3
Rhodothalassium	1		
Riemerella	1331	19	2
Rivibacter	47	78	70
Robiginitomaculum	2		
Roseateles	4	11	
Roseibaca			2
Roseibacterium	2		
Roseicyclus	17	6	
Roseospirillum	3		
Rothia	49	7	2
Rubribacterium	2		
Rubrivivax	12	15	28
Rudaea	5	6	
Rudanella	1		

Género	Cyla	Diba	Pado
Rugamonas	22	40	64
Ruminobacter	40		2
Ruminococcus		2	
Rummeliibacillus		2	6
Saccharibacillus			8
Saccharibacter	12	2	
Saccharococcus	10	5	1
Saccharofermentans	1	2	3
Saccharomonospora	9	1	
Saccharophagus	12		
Saccharopolyspora	1		
Salana		2	1
Salicola	4	10	
Salinarimonas			6
Salinibacterium	6	2	10
Salinicoccus			8
Salinihabitans	1		
Salinimonas	1	1	6
Salirhabdus	13	4	1
Salisaeta	1		
Salmonella	182	12	52
Samsonia	48	16	20
Sandaracinobacter	120	157	93
Sandarakinorhabdus	27	7	6
Sanguibacter	5	1	
Sarcina			2
Schlegelella	1	2	
Schlesneria	3	2	3
Schumannella	8	4	1
Sciscionella	1		
Sebaldella	10 4	7	
Sediminibacterium	5	6	
Sediminimonas		1	1
Segetibacter		5	
Segniliparus	25		
Selenihalanaerobacter	1		
Selenomonas		2	
Seohaeicola	1		
Serpens	47	16	

Appendix 2 Bacterial Genera found in the gut of three wild birds (continuation)

Género	Cyla	Diba	Pado
Serratia	567	322	88
Shimazuella			1
Shimwellia	698	355	19
Shinella	6	7	4
Silanimonas		10	
Silicibacter	5		
Simiduia	111	10	3
Simonsiella	43	25	1
Simplicispira	5	4	12
Singularimonas	4		
Singulisphaera	1	1	2
Sinobacter		1	
Sinorhizobium	82	30	43
Sinosporangium	3		1
Skermanella	3		
Skermania	5		
Smaragdicoccus	233	3	
Sneathia	24	1	
Sodalis	9	5	9
Soehngenia	1		
Solibacillus			1
Solimonas	1		1
Soonwooa	9		
Sorangium	1		
Sphingobacterium	29	5	1
Sphingobium	602	154	4
Sphingomonas	4658	6105	4253
Sphingopyxis	289	463	538
Sphingosinicella	320	553	918
Spirillum	7	20	
Spirochaeta	4	11	
Spongiispira		1	
Sporacetigenium	9		
Sporobacterium	163	4	
Sporocytophaga	2		
Sporolituus		2	
Stakelama	678	572	711
Staphylococcus	54	265	62
Stappia	2		
Starkeya		1	1
Stenotrophomonas	352	203	16
Stenoxybacter	11		
Steroidobacter	1		3
Streptobacillus	4080	52	
Streptococcus	132	246	1583
Streptomyces	1		
Streptophyta	65	83	8
Subtercola	1		
Succinimonas			1
Succinivibrio	2		
Sulfurimonas	1		
Sulfuritalea			2
Suttonella	4		
Syntrophococcus	2		
Tatumella	196	161	21
Telluria	1	1	5
Telmatospirillum	54	112	3
Tepidicella	6	4	1
Tepidimonas			1
Tepidiphilus	1		
Terriglobus			1
Terrimonas	2	4	
Tessaracoccus	2	1	
Thermanaerovibrio	1		
Thermodesulfobium		1	1
Thermodesulforhabdus		2	
Thermogymnomonas	85	3	
Thermoleophilum	1		
Thermomicrobium			2
Thermomonas	1	1	
Thermotalea	1	1	1
Thermus	1		1
Thioalkalispira	1		
Thiobacter		2	1
Thioclava	25	5	2
Thiococcus	1	1	
Thiofaba	1	3	

Appendix 2. Bacterial Genera found in the gut of three wild birds (continuation)

Género	Cyla	Diba	Pado
Thiolamprovum	1		
Thiophaeococcus			1
Thioreductor	1	1	
Thorsellia	11		1
Tistlia		1	
Tistrella	1	1	1
Tolumonas			1
Tomitella	1	1	
Trabulsiella	13	5	17
Trichococcus	1		24
Tropheryma	3	4	2
Tropicimonas	1		
Truepera			1
Tumebacillus		1	
Turicella	50	7	
Turicibacter	6	6	11
Turneriella		1	
Uliginosibacterium		1	
Umboniibacter	3	2	1
Umezawaea	11	1	
Ureaplasma	1		
Ureibacillus		1	
Uruburuella	4	4	
Vagococcus	1	2	1
Vampirovibrio	1	4	
Variovorax	95	142	19
Vasilyevaea	7	2	
Veillonella		7	
Verminephrobacter	3	5	1
Vibrio		5	
Victivallis	1	2	
Vitreoscilla	51		77
Vogesella			11
Vulcanibacillus	1		
Wandonia	1		
Wautersia	18	95	1
Wautersiella	1	6	30
Weeksella	11		
Weissella	1		10
Wenxinia	18		
Wohlfahrtiimonas		1	1
Wolinella	30		
Xanthobacter	1		2
Xanthomonas	20	8	
Xenophilus	2		1
Xenorhabdus		1	
Xylanimicrobium	9	2	1
Xylanimonas	13		
Xylella	3		
Xylophilus	5	3	3
Yeosuana	15		
Yersinia	50	8	1
Yimella	26	5	2
Yokenella	1148	641	188
Yonghaparkia	1	6	
Yuhushiella	51		
Zavarzinella		1	
Zeaxanthinibacter	1		
Zhangella	1		
Zhihengliuella		1	
Zhouia	1	1	
Zimmermannella	18		1
Zobellella	1		1
Zoogloea	3	1	
Zymobacter	6		
Zymomonas	146	167	18
Total	52713	52713	52713