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CORONAVIRUS EN MURCIÉLAGOS NEOTROPICALES EN MÉXICO: PREVALENCIA, FILOGENIA Y COEVOLUCIÓN

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Contenido

Resumen

La diversidad viral puede ser un reflejo de la diversidad de especies. Antes del presente proyecto nada se sabía acerca de la diversidad de coronavirus (CoV) en murciélagos mexicanos, a pesar de la alta diversidad de mamíferos, particularmente de quirópteros, que caracteriza al Neotrópico (que incluye el sur de México). En este trabajo se muestrearon murciélagos del Nuevo Mundo, bajo la hipótesis de que la alta diversidad de quirópteros en esta región revelaría CoVs previamente desconocidos. Los murciélagos son reservorios de una amplia gama de patógenos humanos incluyendo los virus Nipah, Hendra, Ébola, Marburg y distintos CoVs; entre los que destacan aquellos asociados al síndrome respiratorio agudo severo (SARS-CoV) y al síndrome respiratorio del Medio Este (MERS-CoV). Tras el análisis de 1,046 muestras procedentes de 606 murciélagos de 42 especies diferentes capturados en Campeche, Chiapas y la Ciudad de México se identificaron trece CoVs distintos que se dividieron en nueve alfacoronavirus y cuatro betacoronavirus. Doce resultaron nuevos. Se detectó una señal cofilogenética estadísticamente significativa mediante dos pruebas globales de coespeciación, ParaFit y PACo, al analizar los patrones de codivergencia entre las filogenias de murciélagos y CoVs. Los análisis sugieren que estos linajes tienen una historia de diversificación dominada por eventos de coespeciación y que la especiación de los hospederos a nivel de género es un fuerte conductor selectivo en la diversificación de los CoVs. Las asociaciones parásito-hospedero sugieren que el cambio de hospedero es común dentro de murciélagos del mismo género, pero poco frecuente entre individuos de distinto género. La epidemiología de los CoVs es parcialmente influenciada por el origen biogeográfico de las especies hospederas, dado que los CoVs detectados en la zona de transición entre el Neártico y el Neotrópico muestran asociaciones filogenéticas y geográficas específicas. Este modelo representa un marco de trabajo para el estudio de procesos de emergencia viral ligados a factores ecológicos, filogeográficos y evolutivos, el cual es útil para estimar la frecuencia de eventos de cambio de hospedero y potencial emergente para otros virus de RNA con origen en fauna silvestre.

Abstract

Viral diversity can reflect species diversity. Prior to this study nothing was known about the diversity of coronaviruses (CoV) in Mexican bats despite the high diversity of mammals, particularly bats, in the Neotropical region (which includes southern Mexico). In this study New World bats were sampled, under the hypothesis that the high diversity of bats in this region may reveal previously unknown CoVs. Bats are reservoirs for a wide range of human pathogens including Nipah, Hendra, Ebola, Marburg, and the CoVs related to severe acute respiratory syndrome and Middle East respiratory syndrome (SARS- and MERS-CoVs). In a screen of 1,046 samples from 606 bats from 42 different species in Campeche, Chiapas and Mexico City, 13 distinct CoVs were identified. Nine were alpha- and four were beta-CoVs; twelve were novel. We used two global cospeciation tests, ParaFit and PACo, to analyse cophylogenetic patterns between CoVs and chiropteran phylogenies. We detected a significant cophylogenetic signal. The analyses suggest that these lineages have a diversification history dominated by cospeciation events, and that host species at the genus level represent a strong selective driver in CoV diversification. Host-parasite links suggest that host switching is frequent within genera, but rare across bats from different genera. The epidemiology of CoVs is partially influenced by the biogeographical origin of the bat host (i.e. Nearctic or Neotropical), as CoVs detected in the transition zone show specific phylogenetic and geographic associations. This model represents a framework to study zoonotic viral processes linked to ecological, phylogeographic, and evolutionary host traits, suitable to estimate the frequency of host-switching events and spillover potential for other zoonotic RNA viruses of wildlife origin.

Introducción

Panorama mundial

La actividad humana ha alterado los ecosistemas a escala mundial, especialmente en el siglo pasado (Murray & Daszak, 2013). El impacto antropogénico ha ocasionado cambios en la función y estructura de los ecosistemas y en la dinámica de las enfermedades infecciosas, alterando patrones de ocurrencia y distribución de enfermedades, afectando humanos, animales domésticos y animales silvestres (Jones *et al.*, 2008). Un factor clave para entender la aparición de zoonosis emergentes es el reconocimiento de la diversidad viral encontrada en reservorios naturales (Wolfe *et al.*, 2007), especialmente en áreas de alta biodiversidad alteradas por actividades humanas (Daszak *et al.*, 2001) y la relación de esta diversidad viral con factores ecológicos y evolutivos (Daszak *et al.*, 2013; Wood *et al.*, 2012).

La investigación científica acerca de la relación entre la biodiversidad y la dinámica de enfermedades ha tomado gran interés últimamente, generando resultados contrastantes (Keesing *et al.*, 2010; Randolph & Dobson, 2012; Salkeld *et al.*, 2013). Una alta diversidad puede diluir la prevalencia de ciertas enfermedades en algunas interacciones particulares ("efecto de dilución") (Keesing *et al.*, 2010), aunque generalmente este argumento no considera la identidad de los taxones propios de las comunidades de hospederos y patógenos, y este efecto suele depender de la composición específica de las comunidades interactuantes (Randolph & Dobson, 2012). Por otro lado, es bien reconocido que la diversidad biológica puede ser una fuente de nuevos hospederos y parásitos ("efecto de rescate") (Ezenwa *et al.*, 2006; Keesing *et al.*, 2010; Ostfeld, 2009), y se sabe que los agentes patógenos zoonóticos emergentes se correlacionan con la riqueza de especies de mamíferos (Jones *et al.*, 2008), respetando un gradiente latitudinal (Guernier *et al.*, 2004). Además, la riqueza de parásitos ha sido subestimada en

gran medida en prácticamente todos los hospederos vertebrados (Turmelle & Olival, 2009), lo que limita la capacidad predictiva y preventiva en materia de salud pública. Esto sugiere la necesidad de incrementar el número de estudios de exploración enfocados en descubrir la diversidad de parásitos, principalmente en organismos hospederos que sean sospechosos de albergar patógenos potencialmente zoonóticos.

Actualmente, el descubrimiento de patógenos de relevancia para la salud pública y animal se ha convertido en un paradigma en estudios de biodiversidad y salud. Principalmente, se han incrementado los estudios entre la diversidad de murciélagos y la diversidad de diferentes patógenos, especialmente virus zoonóticos de importancia médica (Calisher *et al.*, 2006; Jia *et al.*, 2003; Luis *et al.*, 2013; Plowright *et al.*, 2015; Wood *et al.*, 2012). El interés en virus relacionados a quirópteros deriva del notorio incremento en el número de patógenos emergentes y reemergentes hospedados por estas especies. Al menos 100 especies diferentes de virus han sido aislados o detectados en murciélagos y/o sus tejidos, además, existe evidencia serológica para muchos otros (Turmelle & Olival, 2009).

Los murciélagos han sido señalados como reservorio natural para muchos virus zoonóticos altamente patógenos y carentes de vacunas y/o terapias efectivas actualmente, incluyendo lyssavirus (Freuling *et al.*, 2011), filovirus (Leroy *et al.*, 2005; Towner *et al.*, 2007), flavivirus (Quan *et al.*, 2013) henipavirus (Chua *et al.*, 2000) (Rahman *et al.*, 2010) y otros paramixovirus (Drexler *et al.*, 2012). Uno de los grupos virales más estudiados son los coronavirus (CoVs) recientemente descubiertos como agentes causales de enfermedades zoonóticas emergentes de importancia mundial (Drexler *et al.*, 2014), como el síndrome respiratorio agudo severo (SARS) y el síndrome respiratorio de Oriente Medio (MERS). Este último, ligado a un nuevo betacoronavirus (β-CoV) (Zaki *et al.*, 2012), cuyo surgimiento fue propuesto desde su inicio como un ejemplo más de transmisión zoonótica de fauna silvestre a la población humana. Sin embargo, se necesita más información

de carácter inmunológico, ecológico y evolutivo para confirmar este planteamiento, así como en la mayoría de los patógenos zoonóticos. Esto sugiere que el estudio de los quirópteros desde una perspectiva médico-evolutiva y ecológica sea prioritario.

Panorama nacional

México es considerado un país megadiverso. Es el único país que abarca dos regiones biogeográficas, la Neártica y la Neotropical. La peculiar forma del territorio nacional favorece la convergencia de importantes corredores migratorios para muchos grupos taxonómicos, incluyendo mamíferos, aves y reptiles. A pesar de representar menos del 1% de la superficie terrestre del mundo actual, mantiene cerca del 10 % de la biodiversidad del planeta, incluyendo muchas especies endémicas (Mittermeier *et al.*, 1997). México ostenta el segundo lugar en riqueza de especies de reptiles, tercero en mamíferos, quinto de anfibios y undécimo en riqueza de aves (http://www.conabio.gob.mx). Se han reportado al rededor de 1.8 millones de especies distintas de plantas y animales en México, aunque esta cifra podría estar subestimada pues no contempla ciertos grupos taxonómicos, fundamentalmente, los de microorganismos (Martínez-Meyer *et al.*, 2014). La riqueza natural de México también se manifiesta en la gran diversidad de procesos ecológicos, mismos que han sido alterados de manera dramática por las actividades humanas, particularmente agresivas, de las últimas décadas (Sarukhán *et al.*, 2009). La vegetación natural de México continúa disminuyendo, en 1993 representaba el 54% de la superficie original y en 2002 se redujo a sólo el 38% (Sarukhán *et al.*, 2009). El impacto antropogénico derivado de la modificación de los sistemas naturales y el incontrolado cambio de uso de suelo han modificado la estructura y distribución de la fauna silvestre, reduciendo la biodiversidad y produciendo ambientes que favorecen hospederos, vectores y/o patógenos particulares. La resultante intensificación de las interacciones entre animales silvestres y domésticos incrementa el potencial de transmisión de patógenos previamente desconocidos hacia los animales de producción y la población

humana, estableciendo nuevos ciclos de transmisión (Jones *et al.*, 2013). Es por ello que México es considerado una "zona roja" (*hot spot*) de enfermedades infecciosas emergentes (EIE) de origen zoonótico debido a la distribución de su biodiversidad y la densidad de su población humana (Morse *et al.*, 2012). Hecho que se ha evidenciado a través de brotes recientes de enfermedades humanas y/o de la fauna silvestre, como el virus de la influenza A (H1N1), el virus del Oeste del Nilo y otros flavivirus, alfavirus y hantavirus, entre otros (Mann *et al.*, 2013; Zepeda-Lopez *et al.*, 2010). Estos acontecimientos y su posible relación con la biodiversidad y el cambio de uso de suelo han expuesto la necesidad de estudiar y entender las relaciones entre agentes patógenos, reservorios, vectores y ecosistemas en México.

Diversidad viral

Los virus representan probablemente la fuente más abundante de diversidad genética del planeta. Conducen importantes procesos ecológicos como el ciclo del carbono y moldean las trayectorias evolutivas de los taxones de aquellos hospederos que infectan; hospederos pertenecientes a todos los dominios de la vida, ya que infectan animales, plantas, insectos, hongos, bacterias y arqueas en todos los entornos posibles, de tal suerte, que han jugado un papel primordial en la evolución de todos los organismos (Arias, 2013; Suttle, 2007, 2009). Sin embargo, a pesar de su indudable importancia y de más de 100 años de investigación en el área, el Comité Internacional para la Taxonomía de Virus (ICTV) sólo ha reconocido ~2,600 especies virales (http://www.ictvonline.org). Lo que representa ~0,1 % de los casi 2 millones de especies de animales, plantas y algas que se han descrito a la fecha. La mayoría de los virus que se conocen actualmente han sido descubiertos en seres humanos y animales domésticos, descubrimiento que ha sido enfocado principalmente hacia virus que históricamente han impactado la economía y la salud pública. De hecho, gran parte de lo que sabemos de los virus de fauna silvestre ha surgido a partir de los esfuerzos de investigación, vigilancia y control de EIE en seres humanos, la

mayoría de los cuales provienen de animales (Jones *et al.*, 2008; Levinson *et al.*, 2013). Como consecuencia de su singular mecanismo de replicación viral, los virus de RNA en general y los CoVs en particular, presentan una alta frecuencia de recombinación y elevadas tasas de mutación, lo que les permite adaptarse a hospederos y nichos ecológicos nuevos (Wolfe *et al.*, 2007). Estos y otros datos sugieren que la aparición de enfermedades infecciosas debe considerarse principalmente como un proceso ecológico y es importante estudiarlo desde tal enfoque.

Estudios coevolutivos

Recientemente, los factores relacionados con cambios globales, tales como, la sobrepoblación, el desarrollo tecnológico y económico, la sociedad cada vez más globalizada, el aumento de movilidad y la inconsciente explotación del medio ambiente han contribuido a un aparente aumento en la transmisión de agentes patógenos de animales silvestres a seres humanos (Kuiken *et al.*, 2005). En los últimos años se ha invertido un cuantioso esfuerzo basado en la combinación de diversas disciplinas para descubrir el curso de los acontecimientos relacionados con la introducción de patógenos zoonóticos en la población humana (Anthony *et al.*, 2013b; Daszak *et al.*, 2001; Daszak *et al.*, 2013; Johnson *et al.*, 2013; Keesing *et al.*, 2010; Levinson *et al.*, 2013; Maganga *et al.*, 2014; Rostal *et al.*, 2012; Wolfe, 2005; Wood *et al.*, 2012; Woolhouse *et al.*, 2012). Un enfoque importante para el estudio de patógenos virales se basa en los análisis de coevolución parásito-hospedero. El conjunto virus-hospedero es una condición excepcional para estudiar los procesos coevolutivos, ya que los virus son parásitos intracelulares obligados y su evolución está inexorablemente ligada a las características ecológicas y evolutivas de sus hospederos. El estudio de estas dinámicas coevolutivas puede ser abordado a nivel molecular (Arnaud *et al.*, 2007; Lobo *et al.*, 2009; Pinkert *et al.*, 2011), basado en la continua evolución de la carrera armamentista de acciones virales y contraataques celulares entre el sistema inmune del hospedero y los mecanismos de evasión virales. También es posible

abordarlo mediante perspectivas ecológicas y filogenéticas relacionadas con el proceso macroevolutivo en el que hospederos y virus especian en paralelo debido a la especialización de sus interacciones (Bennett *et al.*, 2014; Lei & Olival, 2014; Lewis-Rogers & Crandall, 2010), o mediante cambios de hospedero (i.e., la mayoría de los patógenos zoonóticos emergentes que han explotado un nuevo hospedero provocando efectos perjudiciales en la mayoría de los casos). Se ha estimado que alrededor de tres cuartas partes de las enfermedades emergentes en seres humanos son resultado de cambios de hospedero de otras especies (Taylor *et al.*, 2001).

Los murciélagos representan uno de los grupos de animales más diversos, tanto taxonómica como funcionalmente (Mayer *et al.*, 2007; Teeling *et al.*, 2005), con un periodo de diversificación que data hasta 85 millones de años antes del presente (Bininda-Emonds *et al.*, 2007; Jones *et al.*, 2005). Poseen características únicas, incluyendo gran longevidad, comportamiento gregario, altas densidades poblacionales, gran movilidad espacial y sistemas inmunes privilegiados (Calisher *et al.*, 2006; Zhang *et al.*, 2013). Muchos de estos rasgos podrían haber contribuido a la capacidad de los murciélagos de coevolucionar con varios microorganismos, y en consecuencia, fungir como reservorios importantes de patógenos zoonóticos (Olival *et al.*, 2012). La diversidad de los virus asociados a ellos sólo ha sido estudiada recientemente (Anthony *et al.*, 2013b); esto a raíz del incremento en la vigilancia epidemiológica en fauna silvestre (Levinson *et al.*, 2013; Wood *et al.*, 2012) y al perfeccionamiento de las técnicas de diagnóstico molecular (Haagmans *et al.*, 2009; Lipkin & Firth, 2013). Con base en las características ecológicas y evolutivas, los CoVs pueden representar a muchos virus zoonóticos de RNA, lo mismo aplica a sus hospederos quirópteros. Estos grupos taxonómicos podrían representar un modelo adaptable al estudio de otras interacciones patógeno-hospedero, por lo que el análisis de sus patrones cofilogenéticos puede aportar información sobre interrogantes básicas acerca de los procesos de emergencia viral.

Antes de la epidemia de SARS la mayoría del conocimiento sobre los CoVs era resultado de investigaciones asociadas a la salud animal, principalmente en animales domésticos, por lo que los aspectos ecológicos y evolutivos han sido poco estudiados. Los estudios filogenéticos y coevolutivos de patógenos emergentes son necesarios para entender su origen, distribución actual, las especies hospederas asociadas y sobre todo proveer de bases para entender la ecología y el potencial zoonótico que representan en diferentes áreas de riesgo. La biodiversidad del sureste de México ofrece el escenario ideal para estudiar relaciones evolutivas entre distintas especies de murciélagos y la diverisdad viral que estos albergan, por lo que el presente proyecto inició en 2012 buscando alcanzar los siguientes objetivos 1) identificar CoVs en murciélagos neotropicales, 2) estudiar la asociación cofilogenética entre especies de murciélagos y CoVs y 3) relacionar la prevalencia y diversidad de virus con los patrones de diversidad de murciélagos en ambientes dominados por el hombre.

Revisión de literatura

En 2002-2003 ocurrió una epidemia asociada a una enfermedad respiratoria severa que se prolongó durante 8 meses, infectó a 8,096 personas y provocó 774 (9.5%) muertes (OMS, 2004), convirtiéndose en la primera pandemia del siglo XXI. El brote de esta enfermedad vulneró los sistemas de salud y económicos internacionales (Bausch & Schwarz, 2014; Beutels *et al.*, 2009; Fonkwo, 2008; Mackey & Liang, 2012), al tiempo que evidenció la fragilidad de los programas de vigilancia epidemiológica a nivel mundial. En 2003 se identificó al SARS-CoV como agente etiológico de la infección humana (Drosten *et al.*, 2003; Ksiazek *et al.*, 2003). A diferencia de los CoVs previamente descritos, mayormente asociados a trastornos respiratorios leves (Van Der Hoek, 2007), este nuevo virus se destacó por ser marcadamente patogénico (Hilgenfeld & Peiris, 2013). Desde el inico del brote se sospechó del origen zoonótico del virus y las primeras investigaciones realizadas en fauna silvestre de China señelaron a las civetas de palma (*Paguma larvata*) como el origen del nuevo CoV (Guan *et al.*, 2003; Xu *et al.*, 2004). Posteriormente, se describió el papel de hospedero intermediario de estos vivérridos en la transmisión zoonótica del SARS-CoV (Song *et al.*, 2005) tras el descubrimiento de CoVs similares al SARS en distintas especies de murciélagos rinolófidos (Lau *et al.*, 2005; Li *et al.*, 2005). El reciente descubrimiento de CoVs en murciélagos de herradura que utilizan el mismo receptor celular que el SARS-CoV humano (Ge *et al.*, 2013) aportó evidencia sobre el papel que juegan como reservorio natural del virus, indicando que no es necesaria la participación de hospederos intermediarios para la infección humana.

En el ámbito veterinario se han identificado y descrito distintos CoVs desde los años 1930s, incluyendo virus altamente patógenos que infectan animales de producción, de laboratorio y mascotas, como el *CoV bovino* (BCoV), el *virus de la gastroenteritis transmisible porcina* (TGEV), el *virus de la bronquitis infecciosa*

aviar (IBV), el *virus de la peritonitis infecciosa felina* (FIPV) y el *virus de la hepatitis murina* (MHV) (Saif, 2004). De manera similar, en los años 1960s se describieron los CoVs humanos HCoV-229E y HCoV-OC43 y posteriormente al brote de SARS, los HCoV-NL63 y HCoV-HKU1 (van der Hoek *et al.*, 2004; Woo *et al.*, 2005). En 2012, un sexto CoV altamente patogénico, identificado como MERS-CoV emergió en Medio Oriente (de Groot *et al.*, 2013; Zaki *et al.*, 2012).

El conocimiento sobre la diversidad de CoVs se ha incrementado de manera significativa desde la pandemia de SARS, con la descripción de varios virus nuevos que afectan una amplia gama de hospederos mamíferos y aves (Cavanagh, 2005; Chu *et al.*, 2011; Dong *et al.*, 2007; Felippe *et al.*, 2010; Guan *et al.*, 2003; Jackwood *et al.*, 2012; Lau *et al.*, 2012b; Woo *et al.*, 2009b; Woo *et al.*, 2009c; Woo *et al.*, 2012). Los murciélagos, en particular, han sido descritos como reservorios importantes de CoVs y llegaron a ser propuestos como hospedero natural de α-CoVs y β-CoVs poco después de la epidemia del SARS (Vijaykrishna *et al.*, 2007; Woo *et al.*, 2009a). Como consecuencia, los esfuerzos de búsqueda se centraron cada vez más en estos animales debido a que los murciélagos continúan siendo reiteradamente señalados como reservorios de un gran número de agentes virales (Calisher *et al.*, 2006; Luis *et al.*, 2013; Olival *et al.*, 2012; Turmelle & Olival, 2009). Las numerosas descripciones de nuevos virus en murciélagos y otros animales silvestres han modificado drásticamente nuestra precepción sobre la relevancia de los reservorios animales en el estudio y entendimiento de las zoonosis emergentes (Karesh *et al.*, 2012; Morse *et al.*, 2012). Sin embargo, el conocimiento sobre el papel de los factores ecológicos y evolutivos que afectan la diversidad viral se descuida frecuentemente. Por ejemplo, se ha demostrado un particular sesgo geográfico referente a los estudios de CoVs en murciélagos, con una evidente falta de información en zonas de alta biodiversidad en África, Asia y América latina (Drexler *et al.*, 2014).

Inicialmente, la mayoría de los estudios orientados al descubrimiento de CoVs en quirópteros fueron dirigidos a murciélagos de China (Chu *et al.*, 2008; Chu *et al.*, 2006; Ge *et al.*, 2012; Ge *et al.*, 2013; He *et al.*, 2014; Lau *et al.*, 2005; Lau *et al.*, 2007; Lau *et al.*, 2010a; Lau *et al.*, 2012a; Lau *et al.*, 2010b; Li *et al.*, 2005; Poon *et al.*, 2005; Ren *et al.*, 2006; Tang *et al.*, 2006; Woo *et al.*, 2007; Woo *et al.*, 2006; Wu *et al.*, 2012; Yang *et al.*, 2014; Yang *et al.*, 2013; Yuan *et al.*, 2010; Yuen *et al.*, 2012), seguidos de una vigilancia limitada en otros países del sudeste asiático, incluyendo Japón, Filipinas, Tailandia, Bangladesh y Arabia Saudita (Anthony *et al.*, 2013b; Gouilh *et al.*, 2011; Memish *et al.*, 2013; Shirato *et al.*, 2012; Suzuki *et al.*, 2014; Tsuda *et al.*, 2012; Wacharapluesadee *et al.*, 2013; Watanabe *et al.*, 2010). En el Viejo Mundo, también se ha descrito una considerable diversidad de nuevos CoVs, tanto en Europa como en África (Annan *et al.*, 2013; August *et al.*, 2012; Balboni *et al.*, 2011; Balboni *et al.*, 2012; De Benedictis *et al.*, 2014; Drexler *et al.*, 2011; Drexler *et al.*, 2010; Falcon *et al.*, 2011; Geldenhuys *et al.*, 2013; Gloza-Rausch *et al.*, 2008; Ithete *et al.*, 2013; Lelli *et al.*, 2013; Pfefferle *et al.*, 2009; Quan *et al.*, 2010; Reusken *et al.*, 2010; Rihtaric *et al.*, 2010; Tao *et al.*, 2012; Tong *et al.*, 2009).

En contraste con la diversidad de CoVs descrita en el Viejo Mundo, se han realizado muy pocas investigaciones en el continente americano y el conocimiento que se tiene acerca de la diversidad de CoVs en este continente antes del 2013 era realmente limitado, especialmente para el caso de la región neotropical. Domínguez y colaboradores (2007) fueron los primeros en muestrear y analizar murciélagos en el Nuevo Mundo en busca de CoVs, seguidos por Donaldson *et al.* (2010) y Osborne *et al.* (2011). Estos grupos examinaron muestras de murciélagos capturados en los estados de Colorado y Maryland, en los EUA, donde encontraron α-CoVs en cinco especies diferentes de murciélagos vespertiliónidos (*Eptesicus fuscus, Myotis evotis, Myotis lucifugus, Myotis occultus* y *Myotis volans*). Todos únicos en comparación con CoVs encontrados en Asia. Posteriormente, Misra *et al.* (2009) analizaron muestras de *M. lucifugus*

capturados en Canadá y detectaron α-CoVs similares a los encontrados en los murciélagos *Myotis* de Colorado. Otras detecciones incluyeron murciélagos vespertiliónidos y molósidos (Huynh *et al.*, 2012; Li *et al.*, 2010). En América del Sur, Carrington *et al*. (2008) identificaron α-CoVs en dos especies de murciélagos de hoja nasal, *Carollia perspicillata* y *Glossophaga soricina*, que genéticamente se relacionan con CoVs detectados en América del Norte y con algunos de los virus hallados en murciélagos europeos. Estos datos resaltan la necesidad de explorar la diversidad y distribución de CoVs en México bajo la hipótesis de que la gran ubicuidad detectada en este grupo de virus y la alta diversidad de especies de murciélagos en la región neotropical de México, permitiría el descubrimiento de diferentes CoVs con características filogenéticas distintas y patrones biogeográficos asociados.

Varios de los nuevos CoVs descritos en la última década fueron identificados en murciélagos de diversas especies y se ha evidenciado una fuerte asociación entre los murciélagos y dichos CoVs (August *et al.*, 2012; Carrington *et al.*, 2008; Chu *et al.*, 2009; Dominguez *et al.*, 2007; Drexler *et al.*, 2011; Drexler *et al.*, 2010; Falcon *et al.*, 2011; Ge *et al.*, 2012; Gloza-Rausch *et al.*, 2008; Li *et al.*, 2005; Misra *et al.*, 2009; Osborne *et al.*, 2011; Pfefferle *et al.*, 2009; Quan *et al.*, 2010; Reusken *et al.*, 2010; Tong *et al.*, 2009; Woo *et al.*, 2006; Yuan *et al.*, 2010). El gran número de CoVs que se siguen detectando en murciélagos sugiere que muchas (si no la mayoría) de las especies de murciélagos podrían estar asociadas con al menos un tipo de CoV. Dado que se han reconocido ~1,200 especies de murciélagos, es factible considerar la existencia de una igualmente amplia diversidad de CoVs.

En 2013, se reportaron 13 nuevos genotipos de CoVs (α-CoVs y β-CoVs) en murciélagos de 4 familias distintas como resultado del presente proyecto (Anthony *et al.*, 2013a). Posteriormente se reportaron hallazgos similares en murciélagos de México, Centro y Suramérica (Corman *et al.*, 2013; Goes *et al.*, 2013; Lima *et al.*, 2013).

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Coronaviruses in bats from Mexico

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Bats are reservoirs for a wide range of human pathogens including Nipah, Hendra, rabies, Ebola, Marburg and severe acute respiratory syndrome coronavirus (CoV). The recent implication of a novel beta (β)-CoV as the cause of fatal respiratory disease in the Middle East emphasizes the importance of surveillance for CoVs that have potential to move from bats into the human population. In a screen of 606 bats from 42 different species in Campeche, Chiapas and Mexico City we identified 13 distinct CoVs. Nine were alpha (α)-CoVs; four were β-CoVs. Twelve were novel. Analyses of these viruses in the context of their hosts and ecological habitat indicated that host species is a strong selective driver in CoV evolution, even in allopatric populations separated by significant geographical distance; and that a single species/genus of bat can contain multiple CoVs. A β-CoV with 96.5% amino acid identity to the β-CoV associated with human disease in the Middle East was found in a *Nyctinomops laticaudatus* bat, suggesting that efforts to identify the viral reservoir should include surveillance of the bat families Molossidae/Vespertilionidae, or the closely related Nycteridae/Emballonuridae. While it is important to investigate unknown viral diversity in bats, it is also important to remember that the majority of viruses they carry will not pose any clinical risk, and bats should not be stigmatized ubiquitously as significant threats to public health.

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INTRODUCTION

Coronaviruses (CoVs), in the subfamily *Coronavirinae*, are enveloped, single-stranded positive-sense RNA viruses with spherical virions of 120–160 nm (King *et al.*, 2012). They are among the largest RNA viruses, with complex polyadenylated genomes of 26–32 kb, and are divided into four genera: *Alphacoronavirus* (α-CoV) and *Betacoronavirus* (β-CoV) (infecting mainly mammals), and *Gammacoronavirus* (γ-CoV) and *Deltacoronavirus* (δ-CoV) (infecting mainly birds) (King *et al.*, 2012; Woo *et al.*, 2012). Infection with CoVs is often asymptomatic, however, they can be responsible for a range of respiratory and enteric diseases of medical and veterinary importance. Chief among these is the severe acute respiratory syndrome (SARS)-CoV, which caused a pandemic in 2002–2003. This outbreak lasted for 8 months, infected 8096 people and resulted in 774 deaths (WHO, 2004). Since then, a renewed public health interest in these viruses has been stimulated by the emergence of a novel β-CoV in nine people from the Middle East. In these cases the patients suffered from acute, serious respiratory illness, presenting with fever, cough, shortness of breath and difficulty breathing (Zaki *et al.*, 2012). Five cases later died.

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The GenBank/EMBL/DDBJ accession numbers for the sequences of CoVs determined in this study are KC117184–KC117213.

It is currently unclear where this particular virus came from, though genomic analyses have shown similarity to bat CoVs (Zaki *et al.*, 2012). Given that the majority of emerging pathogens are known to originate in animals (Jones *et al.*, 2008), the concern is that this current outbreak may represent a further example of zoonotic transmission from wildlife to people, though further ecological, immunological and evolutionary information is still required to confirm this.

Knowledge about CoV diversity has increased significantly since the SARS pandemic, with the description of several novel viruses from a wide range of mammalian and avian hosts (Cavanagh, 2005; Chu *et al.*, 2011; Dong *et al.*, 2007; Felippe *et al.*, 2010; Guan *et al.*, 2003; Jackwood *et al.*, 2012; Lau *et al.*, 2012b; Woo *et al.*, 2009a, b, 2012). Bats in particular seem to be important reservoirs for CoVs, and discovery efforts have been increasingly focused on them since the recognition of SARS-like CoVs in rhinolophid species (Lau *et al.*, 2005; Li *et al.*, 2005) and because bats appear to be reservoirs for a large number of other viruses (Calisher *et al.*, 2006; Drexler *et al.*, 2012; Jia *et al.*, 2003; Leroy *et al.*, 2005; Rahman *et al.*, 2010; Towner *et al.*, 2007). Several of the novel CoVs described in the last decade were identified in bats of various species and demonstrate a strong association between bats and CoVs (August *et al.*, 2012; Carrington *et al.*, 2008; Chu *et al.*, 2009; Dominguez *et al.*, 2007; Drexler *et al.*, 2011, 2010; Falcón *et al.*, 2011; Ge *et al.*, 2012b; Gloza-Rausch *et al.*, 2008; Li *et al.*, 2005; Misra *et al.*, 2009; Osborne *et al.*, 2011; Pfefferle *et al.*, 2009; Quan *et al.*, 2010; Reusken *et al.*, 2010; Tong *et al.*, 2009b; Woo *et al.*, 2006; Yuan *et al.*, 2010).

The large number of CoVs that continue to be described in bats suggest that many (if not most) bat species might be associated with at least one CoV. Given that there are ~1200 extant bat species known, the existence of an equally large diversity of CoVs must be considered likely. Initially, most discovery effort was targeted towards bats from China (Ge *et al.*, 2012a; Lau *et al.*, 2010b; Li *et al.*, 2005; Tang *et al.*, 2006; Woo *et al.*, 2006), followed by limited surveillance in other South-east Asian countries including Japan, the Philippines and Thailand (Gouilh *et al.*, 2011; Shirato *et al.*, 2012; Watanabe *et al.*, 2010). In the Old World, novel CoVs have been found in both Europe and Africa (August *et al.*, 2012; Drexler *et al.*, 2011, 2010; Gloza-Rausch *et al.*, 2008; Pfefferle *et al.*, 2009; Quan *et al.*, 2010; Reusken *et al.*, 2010; Rihtaric *et al.*, 2010; Tong *et al.*, 2009b).

In contrast, very few investigations have been conducted in the New World and little is known about the diversity of CoVs found here. Dominguez *et al.* (2007) were the first to test bats in the New World for CoV, followed by Donaldson *et al.* (2010) and Osborne *et al.* (2011). These groups tested bats captured in Colorado and Maryland and found α-CoVs from five different species of evening bats (*Eptesicus fuscus, Myotis evotis, Myotis lucifugus, Myotis occultus* and *Myotis volans*). All were unique compared with CoVs found in Asia. Misra *et al.* (2009) then tested *M. lucifugus* samples from Canada and detected a similar α-CoV to those found in myotis bats from Colorado. In South America, Carrington *et al.* (2008) identified an α-CoV in two species of leaf-nosed bats, *Carollia perspicillata* and *Glossophaga soricina*, which clustered most closely with CoVs from North American and European bats.

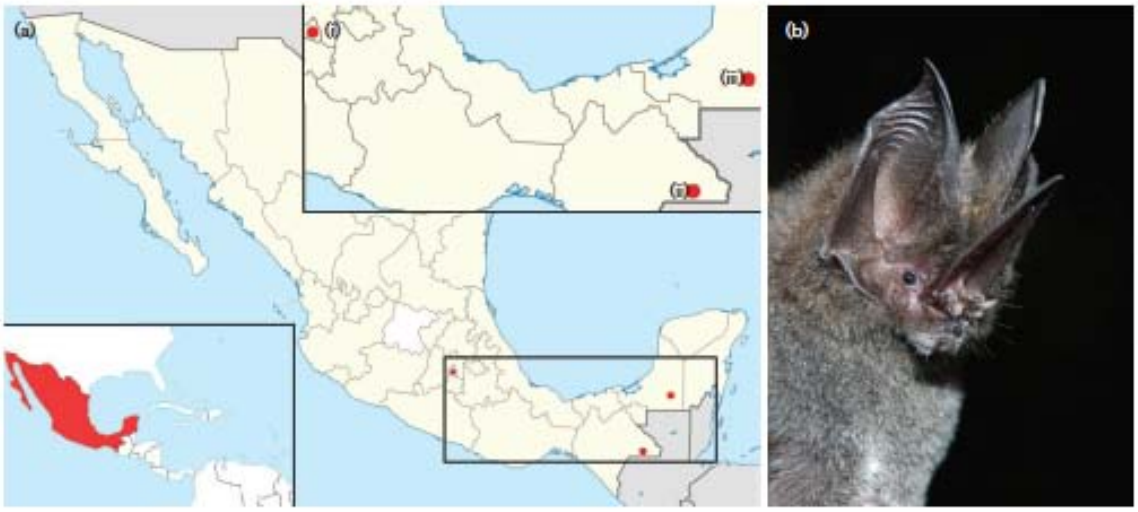

Fig. 1. (a) Map of sampling sites, (i) D.F., Mexico City, (ii) Reserva de la Biosfera Montes Azules, Chiapas, (iii) the Reserva de la Biosfera Calakmul, Campeche. (b) CoV-positive bat, species *Lonchorhina aurita*. This individual (PMX-505) was positive for the novel α-CoV Mex_CoV-3.

Table 1. Summary of all bats captured at each site

A total of 606 bats were sampled across three sites. The number of CoV PCR positives (Pos) are indicated in parentheses, together with the number of CoV clades in square brackets. All bat species captured are endemic to the Americas.

Family	Species	Trophic guild	Campeche		Chiapas		D.F.	Total
			(# CoV PCR Pos)/[CoV clade(s)]		(# CoV PCR Pos)/[# CoV clade(s)]			
			Undisturbed	Disturbed	Undisturbed	Disturbed	Urban	
Phyllostomidae	*Artibeus lituratus*	Frugivorous	26 (4) [5b, 11b]	28 (1) [11a]	16	38		108
	Artibeus phaeotis	Frugivorous	21 (1) [11b]	3	3 (2) [11a, 11b]	9		36
	Artibeus jamaicensis	Frugivorous	33 (2) [5b]	23 (1) [4]	17 (2) [5a]	20		93
	Artibeus watsoni	Frugivorous	5	3	7	2		17
	Glossophaga soricina	Nectivorous	1	8	12	27		48
	Glossophaga commisarisi	Nectivorous			2	1		3
	Carollia sowelli	Frugivorous	27	14 (4) [1, 2, 5b]	11 (1) [5a]	20 (2) [1]		72
	Carollia perspicillata	Frugivorous	8	4 (2) [1]	1	8		21
	Sturnira ludovici	Frugivorous			7	16		23
	Sturnira lilium	Frugivorous	6	6		22		34
	Leptonycteris nivalis	Nectivorous					2	2
	Leptonycteris yerbabuenae	Nectivorous					1	1
	Centurio senex	Frugivorous	1	3				4
	Platyrrhinus helleri	Frugivorous		1	5	10		16
	Uroderma bilobatum	Frugivorous		1	2	2		5
	Desmodus rotundus	Haematophagous			3	4		7
	Micronycteris schmidtorum	Insectivorous			3	2		5
	Micronycteris microtis	Insectivorous			1			1
	Mimon cozumelae	Insectivorous			1			1
	Phyllostomus discolour	Frugivorous				2		2
	Choeroniscus godmani	Nectivorous				6		6
	Trachops cirrhosus	Carnivorous			1	1		2
	Tonatia saurophila	Insectivorous			1			1
	Chrotopterus auritus	Carnivorous			2			2
	Lonchorhina aurita	Insectivorous			1 (1) [3]*			1
	Phylloderma stenops	Frugivorous			2			2
Mormoopidae	*Mormoops megalophyla*	Insectivorous	9			2		11
	Pteronotus davyi	Insectivorous	1		1			2
	Pteronotus parnellii	Insectivorous	1 (1) [10]	1	10	7		19
Molossidae	*Nyctinomops macrotis*	Insectivorous					10	10
	Nyctinomops laticaudatus	Insectivorous	5 (1) [9]					5
	Tadarida brasiliensis	Insectivorous					10 (3) [8]	10
Vespertilionidae	*Myotis velifer*	Insectivorous					7 (3) [7]	7
	Myotis occultus	Insectivorous					1	1

Table 1. cont.

Family	Species	Trophic guild	Campeche		Chiapas		D.F.	Total
			(# CoV PCR Pos)/[CoV clade(s)]		(# CoV PCR Pos)/[# CoV clade(s)]			
			Undisturbed	Disturbed	Undisturbed	Disturbed	Urban	
	Myotis keaysi	Insectivorous			2			2
	Myotis nigricans	Insectivorous			1			1
	Eptesicus fuscus	Insectivorous				1 (1) [6]	2	3
	Corynorhinus mexicanus	Insectivorous					1	1
	Lasiurus intermedius	Insectivorous		1				1
	Bauerus dubiaquercus	Insectivorous			17	1		18
Emballonuridae	*Rhynchonycteris naso*	Insectivorous			1			1
	Saccopteryx bilineata	Insectivorous				1		1
	Total species/families		13 [9, 10, 5b, 11b]	13 [1, 2, 4, 5b, 11a]	26 [3, 5a, 11a, 11b]	22 [1, 6]	8 [7, 8]	
	Total animals captured		144	96	130	202	34	606

*Individual PMX-505/*Leptonycteris curasoae* (Fig. 1b).

Nothing is known about the diversity of CoVs in Mexico. Many of the bats studied in Canada and the USA are also found in Mexico, yet it is unknown whether similar viruses are found here. It is also unknown whether β-CoVs exist in the Americas, or whether the α-CoVs predominate. This is a substantive gap in our knowledge of CoV ecology because one-third of all bat species (and 75% of all known bat genera) are found in the neotropics, which includes southern Mexico (Osborne *et al.*, 2011; Wilson & Reeder, 2005). It seems probable that the high ecological, trophic and taxonomic diversity found in Neotropical and Nearctic bats in Mexico (Arita & Ortega, 1998) would be matched by an equally diverse population of novel CoVs. In this study we examined 42 species of bats using broadly reactive consensus PCR for the discovery of novel CoVs, and found an additional 13 viral lineages/clades, clustering in both the α-CoV and the β-CoV genera. Phylogenetic analysis of these new viruses has provided insight into the molecular epidemiology of CoVs, and shows that host speciation is a significant driver in CoV evolution.

RESULTS AND DISCUSSION

The goal of this study was to increase our knowledge of CoV diversity in bats from southern Mexico. Three sites were included in the study: Campeche, Chiapas and Mexico City (Mexico Distrito Federal; D.F.) (Fig. 1). At two of the sites (Chiapas and Campeche) bats were captured in disturbed and undisturbed habitat to investigate how anthropogenic activity may affect host and viral diversity. Such habitat gradients do not exist in D.F., which is a highly urbanized site. A total of 1046 samples were collected from 606 individuals, of 42 different bat species (Table 1).

Host (bat) diversity

Host diversity was examined at all sites. In Chiapas, a species richness of 32 was recorded, and the calculated Shannon–Wiener diversity index (H′) was 2.81 (Table 2). A comparison of undisturbed and disturbed habitat in Chiapas (Shannon *t*-test) revealed no significant difference in richness and diversity (P=0.11). In Campeche the overall species richness was 16 and the diversity index H′ was 2.167 (Table 2). Again, no significant difference in host diversity was seen between the undisturbed and disturbed habitats (P=0.44). Previous work has shown that bat diversity often reflects the level of disturbance for a given habitat, with lower diversity recorded in disturbed areas (Medellín *et al.*, 2000). No such distinction was observed here between disturbed and undisturbed sites. This may reflect the dominance of bats from the genera *Artibeus* and *Carollia* (Table 1), both of which contain species that are known to be more adaptable and resistant to the effects of habitat fragmentation (Medellín *et al.*, 2000). An increased sampling effort including larger spatial and temporal scales will be needed to assess whether the abundance and

richness of less-well represented species alter the overall bat diversity in each fragment. In D.F. (Mexico City), eight species were captured and the diversity index H′ was 1.69 (Table 2). Sampling effort was not consistent among the three sites, precluding any direct comparisons of diversity between Chiapas, Campeche and D.F.

CoV diversity

Broadly reactive consensus PCR revealed CoV sequences in 32/606 (5.3 %) bats (Table 1). Sequence analyses indicated high phylogenetic diversity and the presence of 13 distinct clades at the nucleotide level (Fig. 2). Clades 5a/5b and 11a/11b had high nucleotide sequence identity and collapsed into a single group when analysed at the amino acid level (data not shown). Nine of the viruses clustered with known α-CoVs, and four clustered with β-CoVs (Fig. 2). One of the α-CoVs (Mex_Cov-6) was closely related to a virus identified previously in an *Eptesicus fuscus* bat, sampled on the Appalachian Trail in Maryland, USA (Donaldson *et al.*, 2010). We therefore extend the known geographical range of this virus to south-eastern Mexico and present the discovery of a further 12 novel CoVs.

Prior to this study, very little was known about the diversity of CoVs in the neotropics, despite the high diversity of bat species found here (Wilson & Reeder, 2005). Here, we demonstrate that several additional viruses from both the genus *Alphacoronavirus* and the genus *Betacoronavirus* exist in Mexico. This particular study was limited to the analysis of a 329 bp fragment of the RNA-dependent RNA polymerase (RdRp), however, it was sufficient for the identification of these novel strains and therefore satisfied our primary goal of discovery.

CoV-positive sample types included 27 rectal swabs, four oral swabs and one blood sample (annotated on Fig. 2). The high number of positive rectal samples agrees with previous studies, which showed CoV detection in bats to be almost exclusively restricted to faeces (Lau *et al.*, 2005; Li *et al.*, 2005; Pfefferle *et al.*, 2009; Tang *et al.*, 2006). Detection in oral swabs has also been demonstrated, but much less frequently (Carrington *et al.*, 2008).

Phylogenetic analyses of this short fragment show that CoVs cluster based on the relatedness of host species. Fig. 2 shows that all of the α-CoVs detected in phyllostomid bats cluster together; as do all α-CoVs discovered in miniopterid bats. The families Vespertilionidae and Molossidae are closely related (Agnarsson *et al.*, 2011; Teeling *et al.*, 2005), and viruses from these bats also cluster together, though the additional presence of CoV HKU2 (from a rhinolophid bat; Woo *et al.*, 2006) in this group is currently unexplained. In the β-CoV genus a similar pattern is observed. All viruses identified in rhinolophid bats cluster together, as do viruses from the vespertilionid/molossid group, and equally so in the related mormoopid/phyllostomid group. These results suggest purifying selection, which is apparently effective at the level of host species or genus. For example, the α-CoV Mex_CoV-1 was only found in *Carollia* spp. bats, but could be present variably in the species *Carollia sowelli* or *Carollia perspicillata*. The same is true of the β-CoVs Mex_CoV-11a and Mex_CoV-11b, both of which were only found in *Artibeus* spp. bats, but which could be present in either *Artibeus lituratus* or *Artibeus phaeotis*. Mex_CoV-6 was found in an *Eptesicus* sp. bat and clustered very closely with the previously identified *Eptesicus*-associated CoV (GenBank accession no. HQ585086). Finally, the close association of Mex-CoV-7 and -8 with *Myotis velifer* and *Tadarida brasiliensis*, respectively, also suggest strong host specificity. These results agree with previous studies that show individual CoVs are associated with a single species or genus, even among co-roosting species – including *Miniopterus*, *Rousettus*, *Rhinolophus* and *Hipposideros* bats (Chu *et al.*, 2006; Drexler *et al.*, 2010; Gouilh *et al.*, 2011; Pfefferle *et al.*, 2009; Quan *et al.*, 2010; Tang *et al.*, 2006).

Phylogenetic association of CoVs with host species/genus is particularly evident in allopatric populations separated by significant geographical distances; such as Mex_CoV-6 and the previously identified HQ585086 (GenBank accession no.) virus from Maryland, both of which were found in *Eptesicus fuscus* and shared a very high sequence identity despite being separated by >2500 km. Misra *et al.* (2009) reported a similar observation in North America, noting highly similar viruses from *Myotis* spp. bats in Canada and Colorado. And the same appears to be true in *Myotis ricketti* in Asia (Tang *et al.*, 2006), in *Chaerephon* and *Rousettus* in Africa (Tong *et al.*, 2009b), and in *Nyctalus* and *Myotis* spp. bats in Europe (August *et al.*, 2012; Drexler *et al.*, 2010; Gloza-Rausch *et al.*, 2008). In all cases it was concluded that even if populations of these species were thousands of kilometres apart, highly similar CoVs could be detected. It is important to qualify that our results are based on a short sequence, and additional studies will be required to assess whether our observations are consistent when additional sequences from other genes/proteins are considered.

Mex_CoV-5b was the only virus to be found in two distinct (but related) genera, having been detected in both *Artibeus* and *Carollia* bats (Fig. 2). Such findings have been reported previously, albeit rarely (Lau *et al.*, 2012a; Osborne *et al.*, 2011; Tong *et al.*, 2009a), and demonstrate that CoVs can infect individuals from different genera/suborders. It is interesting to note that this particular bat (*Carollia sowelli*, PMX-1232) was captured in a disturbed habitat. Increased efforts for viral discovery in this region will be required to investigate whether disturbed habitats provide increased risk or opportunity for viruses to spillover into new species, as previously suggested (Cottontail *et al.*, 2009; Keesing *et al.*, 2010; Suzán *et al.*, 2012). That said, the health risk to people probably remains low, and bats should not be viewed as a liability, especially given the vital ecosystem functions they serve (Medellín, 2009).

Strong associations of CoVs with host species/genus could prove to be extremely useful in identifying potential

Table 2. Evaluations of host and viral diversity at each site/habitat using the Shannon diversity index

This index takes into account the number of individuals as well as the number of taxa. A 0 value means that community has only a single taxon.

Site	Habitat	Bat diversity			Viral diversity		
		n	S (richness)	D (diversity)	*n*	S (richness)	D (diversity)
Chiapas	Undisturbed	130	26	2.775	6	5	1.561
	Disturbed	202	22	2.57	3	2	0.6365
	Total	**332**	**32**	**2.81**	**9**	**6**	**1.735**
Campeche	Undisturbed	144	13	2.079	9	4	1.149
	Disturbed	96	13	2.01	8	5	1.494
	Total	**240**	**16**	**2.167**	**17**	**7**	**1.732**
D.F.	Urban	34	8	1.69	6	2	0.6931
	606	42		32	13		606

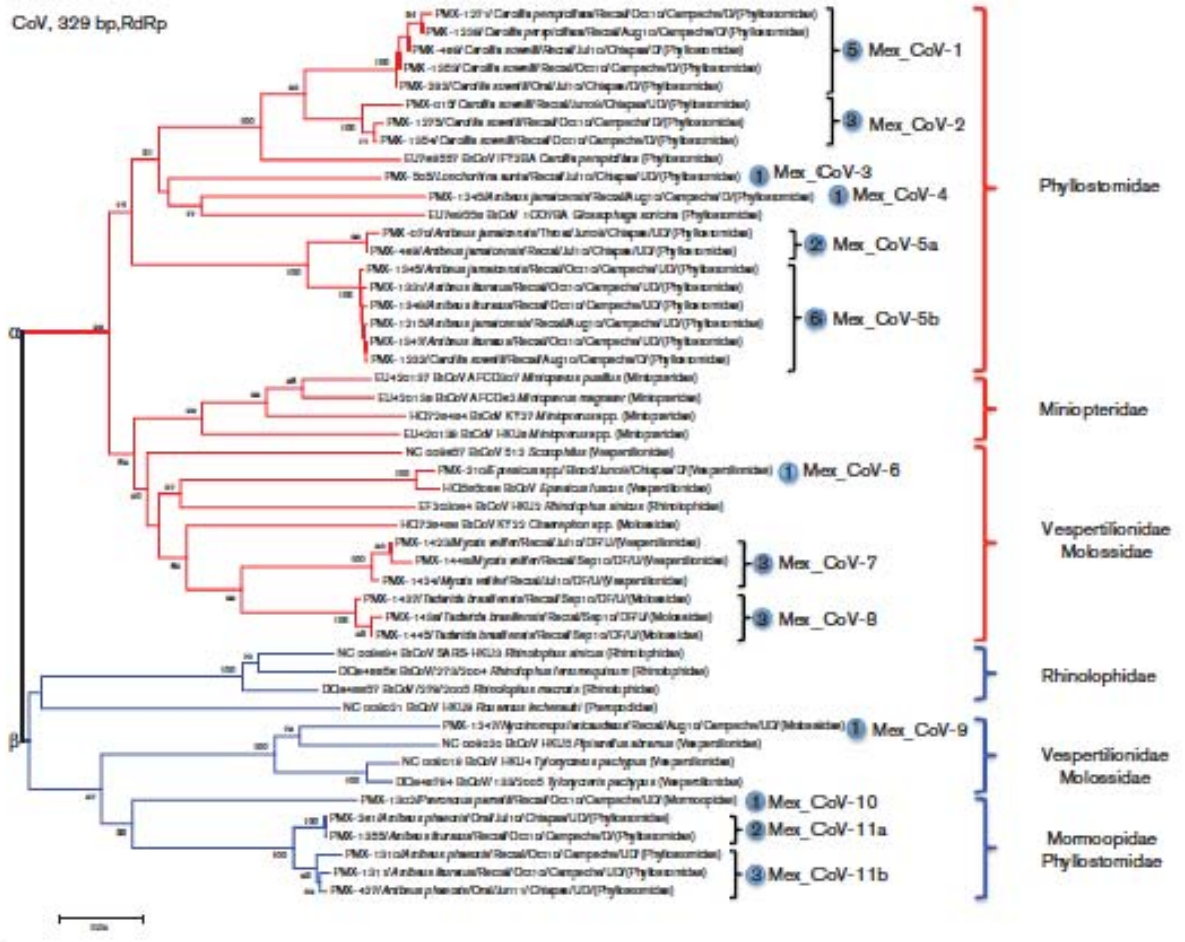

Fig. 2. Maximum-likelihood tree of a 329 bp fragment of the RdRp from bat CoVs only (red, α-CoVs; blue, β-CoVs). All 32 positive animals from this study are presented on the tree, and begin with a PMX number that refers to the animal identity. The species of all PMX animals was confirmed by Cyt-b (cytochrome b gene) barcoding. CoVs identified in this study split into 13 clades at the nucleotide level, though Mex_CoV-5a/b and Mex_CoV-11a/b collapse into single clades when analysed at the amino acid level. Each clade is indicated by a blue circle, and the total number of positive animals for each clade is indicated within. D, Disturbed habitat; UD, undisturbed habitat; U, urban habitat. Bar, 0.05 nucleotide substitutions per site.

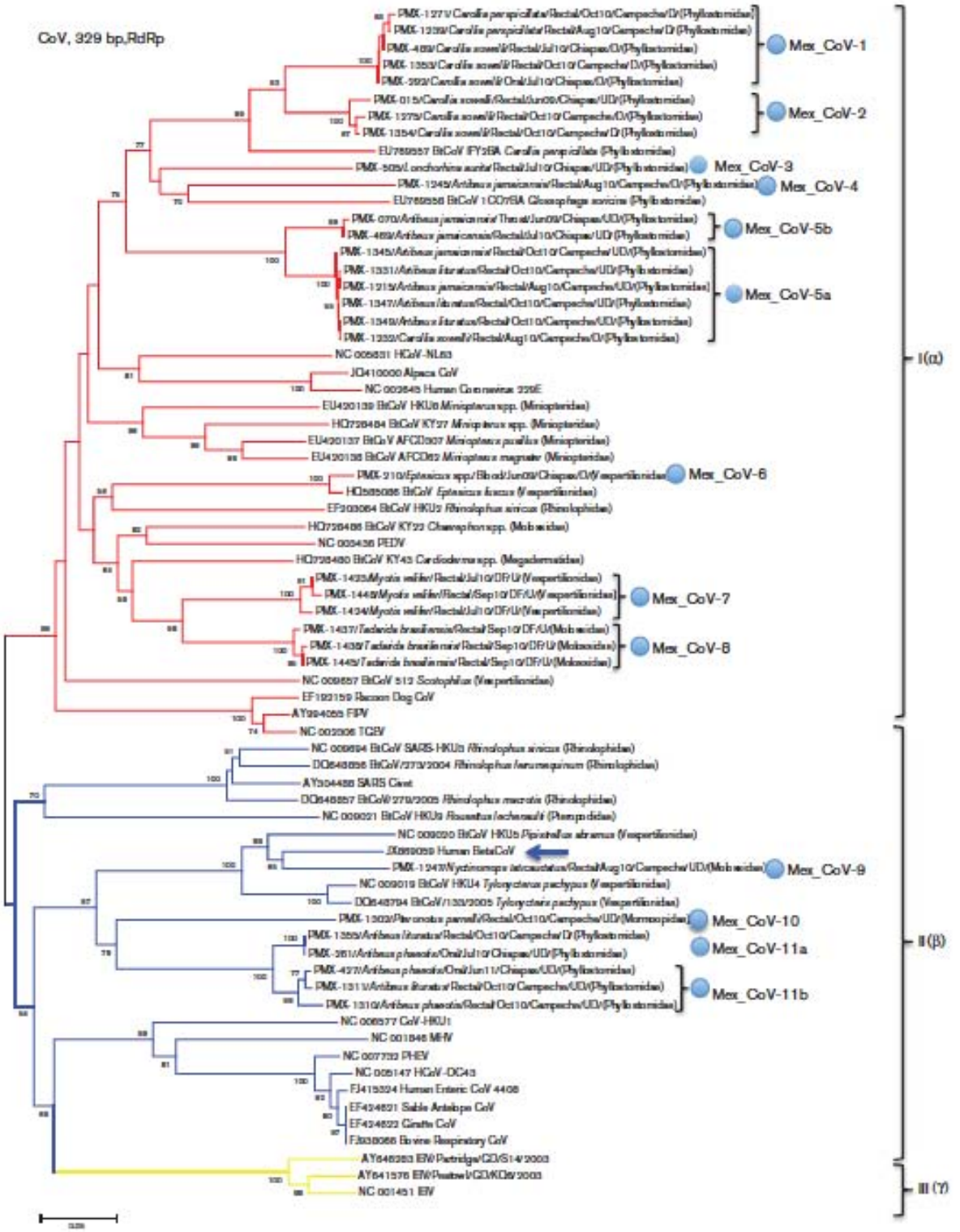
CoV, 329 bp,RdRp
Mex_CoV-1
Mex_CoV-2
Mex_CoV-3
Mex_CoV-4
Mex_CoV-5b
Mex_CoV-5a
Mex_CoV-6
Mex_CoV-7
Mex_CoV-8
Mex_CoV-9
Mex_CoV-10
Mex_CoV-11a
Mex_CoV-11b
I(α)
II(β)
III (γ)

Fig. 3. Maximum-likelihood tree of a 329 bp fragment of the RdRp from all CoVs (red, α-CoVs; blue, β-CoVs; yellow, γ-CoVs). Viruses discovered in this study are indicated by blue circles. The 2012 human β-CoV is indicated by an arrow, and clusters most closely to PMX-1247/*Nyctinomops laticaudatus*. Bar, 0.05 nucleotide substitutions per site.

reservoirs for viruses that do spillover into other species, assuming that an emergent virus still shares sufficient similarity to those circulating in the original host. A phylogenetic analysis of the new human β-CoV that recently emerged in Saudi Arabia showed that the virus clusters with viruses from bats in the vespertilionid/molossid families, and that the closest relative is the Mex_CoV-9 virus that was identified in a *Nyctinomops laticaudatus* bat from this study (Fig. 3). Sequence identity between these two viruses is 86.5 % at the nucleotide level, but 96.5 % at the amino acid level. When only the first and second nucleotide positions are considered, nucleotide identity jumps to 97.1 %, demonstrating both that there is strong purifying selection acting on these viruses, and that Mex_CoV-9 and human β-CoV have probably been evolving separately for quite some time. These results do not mean the Saudi Arabian CoV originates in *Nyctinomops* spp. bat, but do suggest that any search for the original reservoir of this virus should perhaps focus on bats in the molossid/vespertilionid families, or the related nycterid/emballonurid families.

Artibeus was the only genus shown to contain more than one CoV, with the detection of the α-CoVs Mex_CoV-5a and 5b, Mex_CoV-4, and the β-CoVs Mex_CoV-11a/b. However, bats in this genus were also the most frequently sampled (Table 1), which probably explains why more viruses were identified. Other studies have also identified multiple CoVs within a single species, including *Rhinolophus sinicus* (Yuan *et al.*, 2010) and *Rousettus leschenaulti* in China (Lau *et al.*, 2010a), and *Miniopterus* spp. and *Rhinolophus* spp. from Europe (Drexler *et al.*, 2010); all of which were shown to be doubly and triply infected with different CoVs. Further studies focusing on rarely represented species would be needed to investigate whether population size determines the ability for bats to carry more than one CoV, or whether all genera are capable of supporting multiple CoVs independently of population size. In this study, when multiple CoVs were discovered in a given bat species/genus they were often closely related, for example α-CoVs Mex_CoV-5a/b and β-CoVs Mex_CoV-11a/b. When examined at the amino acid level, these clades collapsed into single groups, yet they maintained separate clades at the nucleotide level, suggesting the contemporary evolution of new strains in these bats. Together, these results highlight the importance of screening sufficient numbers of individuals per species when attempting to describe viral diversity in a given population/region.

Methods used to assess the diversity of host species were also used to measure CoV diversity at undisturbed and disturbed sites in Chiapas and Campeche. No significant difference was seen in CoV diversity across gradients (Chiapas, $P=0.10$; Campeche, $P=0.47$), mirroring the non-significant difference also observed in the host (described above).

METHODS

Sites and sampling. Bats were captured at three different sites in Mexico, the Reserva de la Biosfera Montes Azules (Chiapas), the Reserva de la Biosfera Calakmul (Campeche), and Mexico City (D.F., Fig. 1). The first two sites, located in south-eastern Mexico represent regions of high species diversity, and are characterized by large tracts of continuous primary vegetation, while Mexico City represents a highly urbanized site. In Chiapas and Campeche bats were collected in two landscape gradients, assigned as: (1) 'Undisturbed' forest (UD), where any sign of human impact is largely absent; and (2) 'Disturbed' (D), defined as the transition zone between areas of primary vegetation and agriculture/livestock, and by the presence of urban areas. Landscape units were separated by at least 10 km. Capture effort included two nights of trapping using 5 ×9 m mist nets by roosts or foraging sites. Nets were opened at dusk and remained open for 4 h consecutively. Identification of animals was made using field guides (Medellín *et al.*, 2008). Oral and rectal swabs, and blood were collected (when possible) from each animal. For blood samples <10% of the blood volume was collected and for small bats blood was taken using protocols previously described (Smith *et al.*, 2010). A veterinarian was present for all sampling and all animals were released safely at the site of capture. Samples were collected directly into lysis buffer and preserved at −80 °C until transfer to the Center for Infection and Immunity for CoV screening. Capture and sample collection was approved by the Institutional Animal Care and Use Committee at the University of California, Davis (protocol number: 16048).

Laboratory testing. Total nucleic acid was extracted from all samples using the EasyMag (bioMérieux) platform, and cDNA synthesis performed using SuperScript III first strand synthesis supermix (Invitrogen), all according the manufacturer's instructions. CoV discovery was performed using broadly reactive consensus PCR primers, targeting the RdRp (Quan *et al.*, 2010). PCR products of the expected size were cloned into Stratuclone PCR cloning vector and sequenced using standard M13R primers. If an individual tested positive for CoV, the species of the bat was secondarily confirmed with genetic barcoding, targeting both cytochrome oxidase subunit I and *Cyt-b* mitochondrial genes, as described previously (Townzen *et al.*, 2008).

Analysis. Sequences were edited using Geneious Pro (5.6.4). Alignments were constructed using CLUSTAL W, executed through Geneious, and refined manually. Neighbour-joining and maximum-likelihood trees were built in MEGA (5.0), and bootstrapped using 1000 repetitions. Nucleotide trees that represent a consensus of both methods are presented. Evaluations of host and viral diversity at each site/habitat were made using the Shannon–Wiener diversity index (H') using the Past 1.81 software. This index takes into account the number of individuals as well as the number of taxa. A 0 value means that the community has only a single taxon. Comparison of the Shannon–Wiener diversities (entropies) were calculated between habitat types for each region using the Shannon *t*-test, described by Poole (1974).

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REFERENCES

Agnarsson, I., Zambrana-Torrelio, C. M., Flores-Saldana, N. P. & May-Collado, L. J. (2011). A time-calibrated species-level phylogeny of bats (Chiroptera, Mammalia). *PloS Currents: Tree of Life* **Version 44.**

Arita, H. T. & Ortega, J. (1998). The Middle-American bat fauna: conservation in the Neotropical-Nearctic border. In *Bat Biology and Conservation*, pp. 295–308. Edited by T. H. Kunz & P. A. Racey. Washington, DC: Smithsonian Institution Press.

August, T. A., Mathews, F. & Nunn, M. A. (2012). Alphacoronavirus detected in bats in the United Kingdom. *Vector Borne Zoonotic Dis* 12, 530–533.

Calisher, C. H., Childs, J. E., Field, H. E., Holmes, K. V. & Schountz, T. (2006). Bats: important reservoir hosts of emerging viruses. *Clin Microbiol Rev* 19, 531–545.

Carrington, C. V., Foster, J. E., Zhu, H. C., Zhang, J. X., Smith, G. J., Thompson, N., Auguste, A. J., Ramkissoon, V., Adesiyun, A. A. & Guan, Y. (2008). Detection and phylogenetic analysis of group 1 coronaviruses in South American bats. *Emerg Infect Dis* 14, 1890–1893.

Cavanagh, D. (2005). Coronaviruses in poultry and other birds. *Avian Pathol* 34, 439–448.

Chu, D. K. W., Poon, L. L. M., Chan, K. H., Chen, H., Guan, Y., Yuen, K. Y. & Peiris, J. S. M. (2006). Coronaviruses in bent-winged bats (*Miniopterus* spp.). *J Gen Virol* 87, 2461–2466.

Chu, D. K. W., Peiris, J. S. M. & Poon, L. L. M. (2009). Novel coronaviruses and astroviruses in bats. *Virol Sin* 24, 100–104.

Chu, D. K. W., Leung, C. Y. H., Gilbert, M., Joyner, P. H., Ng, E. M., Tse, T. M., Guan, Y., Peiris, J. S. M. & Poon, L. L. M. (2011). Avian coronavirus in wild aquatic birds. *J Virol* 85, 12815–12820.

Cottontail, V. M., Wellinghausen, N. & Kalko, E. K. (2009). Habitat fragmentation and haemoparasites in the common fruit bat, *Artibeus jamaicensis* (Phyllostomidae) in a tropical lowland forest in Panamá. *Parasitology* 136, 1133–1145.

Dominguez, S. R., O'Shea, T. J., Oko, L. M. & Holmes, K. V. (2007). Detection of group 1 coronaviruses in bats in North America. *Emerg Infect Dis* 13, 1295–1300.

Donaldson, E. F., Haskew, A. N., Gates, J. E., Huynh, J., Moore, C. J. & Frieman, M. B. (2010). Metagenomic analysis of the viromes of three North American bat species: viral diversity among different bat species that share a common habitat. *J Virol* 84, 13004–13018.

Dong, B. Q., Liu, W., Fan, X. H., Vijaykrishna, D., Tang, X. C., Gao, F., Li, L. F., Li, G. J., Zhang, J. X. & other authors (2007). Detection of a novel and highly divergent coronavirus from Asian leopard cats and Chinese ferret badgers in Southern China. *J Virol* 81, 6920–6926.

Drexler, J. F., Gloza-Rausch, F., Glende, J., Corman, V. M., Muth, D., Goettsche, M., Seebens, A., Niedrig, M., Pfefferle, S. & other authors (2010). Genomic characterization of severe acute respiratory syndrome-related coronavirus in European bats and classification of coronaviruses based on partial RNA-dependent RNA polymerase gene sequences. *J Virol* 84, 11336–11349.

Drexler, J. F., Corman, V. M., Wegner, T., Tateno, A. F., Zerbinati, R. M., Gloza-Rausch, F., Seebens, A., Müller, M. A. & Drosten, C. (2011). Amplification of emerging viruses in a bat colony. *Emerg Infect Dis* 17, 449–456.

Drexler, J. F., Corman, V. M., Müller, M. A., Maganga, G. D., Vallo, P., Binger, T., Gloza-Rausch, F., Rasche, A., Yordanov, S. & other authors (2012). Bats host major mammalian paramyxoviruses. *Nat Commun* 3, 796.

Falcón, A., Vázquez-Morón, S., Casas, I., Aznar, C., Ruiz, G., Pozo, F., Perez-Breña, P., Juste, J., Ibáñez, C. & other authors (2011). Detection of alpha and betacoronaviruses in multiple Iberian bat species. *Arch Virol* 156, 1883–1890.

Felippe, P. A. N., da Silva, L. H. A., Santos, M. M., Spilki, F. R. & Arns, C. W. (2010). Genetic diversity of avian infectious bronchitis virus isolated from domestic chicken flocks and coronaviruses from feral pigeons in Brazil between 2003 and 2009. *Avian Dis* 54, 1191–1196.

Ge, X., Li, Y., Yang, X., Zhang, H., Zhou, P., Zhang, Y. & Shi, Z. (2012a). Metagenomic analysis of viruses from bat fecal samples reveals many novel viruses in insectivorous bats in China. *J Virol* 86, 4620–4630.

Ge, X., Li, Y., Yang, X., Zhang, H., Zhou, P., Zhang, Y. & Shi, Z. (2012b). Metagenomic analysis of viruses from bat fecal samples reveals many novel viruses in insectivorous bats in China. *J Virol* 86, 4620–4630.

Gloza-Rausch, F., Ipsen, A., Seebens, A., Göttsche, M., Panning, M., Drexler, J. F., Petersen, N., Annan, A., Grywna, K. & other authors (2008). Detection and prevalence patterns of group I coronaviruses in bats, northern Germany. *Emerg Infect Dis* 14, 626–631.

Gouilh, M. A., Puechmaille, S. J., Gonzalez, J.-P., Teeling, E., Kittayapong, P. & Manuguerra, J.-C. (2011). SARS-Coronavirus ancestor's foot-prints in South-East Asian bat colonies and the refuge theory. *Infect Genet Evol* 11, 1690–1702.

Guan, Y., Zheng, B. J., He, Y. Q., Liu, X. L., Zhuang, Z. X., Cheung, C. L., Luo, S. W., Li, P. H., Zhang, L. J. & other authors (2003). Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science* 302, 276–278.

Jackwood, M. W., Hall, D. & Handel, A. (2012). Molecular evolution and emergence of avian gammacoronaviruses. *Infect Genet Evol* 12, 1305–1311.

Jia, G. L., Zhang, Y., Wu, T. H., Zhang, S. Y. & Wang, Y. N. (2003). Fruit bats as a natural reservoir of zoonotic viruses. *Chin Sci Bull* 48, 1179–1182.

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L. & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature* 451, 990–993.

Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., Hudson, P., Jolles, A., Jones, K. E. & other authors (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature* 468, 647–652.

King, A. M. Q., Adams, M. J., Carstens, E. B. & Lefkowitz, E. J. (editors) (2012). *Virus Taxonomy: Classification and Nomenclature of Viruses. Ninth Report of the International Committee on Taxonomy of Viruses.* San Diego, CA: Elsevier.

Lau, S. K., Woo, P. C., Li, K. S., Huang, Y., Tsoi, H. W., Wong, B. H., Wong, S. S., Leung, S. Y., Chan, K. H. & Yuen, K. Y. (2005). Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. *Proc Natl Acad Sci U S A* 102, 14040–14045.

Woo, P. C. Y., Lau, S. K. P., Lam, C. S. F., Lai, K. K. Y., Huang, Y., Lee, P., Luk, G. S. M., Dyrting, K. C., Chan, K. H. & Yuen, K. Y. (2009b). Comparative analysis of complete genome sequences of three avian coronaviruses reveals a novel group 3c coronavirus. *J Virol* 83, 908–917.

Woo, P. C. Y., Lau, S. K. P., Lam, C. S. F., Lau, C. C. Y., Tsang, A. K. L., Lau, J. H. N., Bai, R., Teng, J. L. L., Tsang, C. C. C. & other authors (2012). Discovery of seven novel mammalian and avian coronaviruses in the genus *Deltacoronavirus* supports bat coronaviruses as the gene source of *Alphacoronavirus* and *Betacoronavirus* and avian coronaviruses as the gene source of *Gammacoronavirus* and *Deltacoronavirus*. *J Virol* 86, 3995–4008.

Yuan, J. F., Hon, C. C., Li, Y., Wang, D. M., Xu, G. L., Zhang, H. J., Zhou, P., Poon, L. L. M., Lam, T. T. Y. & other authors (2010). Intraspecies diversity of SARS-like coronaviruses in *Rhinolophus sinicus* and its implications for the origin of SARS coronaviruses in humans. *J Gen Virol* 91, 1058–1062.

Zaki, A. M., van Boheemen, S., Bestebroer, T. M., Osterhaus, A. D. & Fouchier, R. A. (2012). Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med* 367, 1814–1820.

Bat-Coronavirus co-phylogenetic patterns in Mexico indicate strong host specificity

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Abstract

Host range is believed to be an important risk factor for zoonotic emergence. Viruses that can infect multiple hosts are considered more likely to emerge as pathogens, compared with those that are restricted to a single host species or to a group of closely related hosts. Understanding co-phylogenetic patterns between coronaviruses (CoVs) and their hosts is therefore of interest because of the potential to reveal patterns in the degree of specificity for different host species and geographical regions. Here we tested for congruence between alpha(α)-CoVs and beta(β)-CoVs and their bat hosts in Mexico, using global co-speciation tests, ParaFit and PACo. We found evidence for significant congruence between host and viral phylogenies suggesting that some degree of co-speciation has occurred in this region. In contrast, a related study on bats from China did not identify co-phylogenetic congruence. We therefore suggest that there may be geographical variation in the degree of host specificity for CoVs, which may subsequently reflect regional differences in pathogen emergence risk.

Introduction

Bats have been proposed as a natural reservoir for many important zoonotic viruses, including lyssa-, filo-, flavi-, paramyxo-, and corona- viruses (CoVs) [1-5]. In recent years, factors such as land-use change, population growth and increased trade and travel have contributed to an increase in the emergence of such viruses from wildlife to humans [6]; this has led to greater interdisciplinary efforts to understand the course of events related to zoonotic emergence [7-9]. One approach is the reconstruction of host and viral phylogenies to look for congruence in evolutionary histories, and where possible to identify host-specialists and host-generalists (i.e. higher likelihood of spillover). During the last decade, several such ‘co-phylogenetic’ studies have focused on viruses from different families [10-15], as well as parasites [16,17]. These studies have shown contrasting patterns in the congruence of host:microbe phylogenies, and have suggested that the degree of

association can be influenced by both microbial factors (e.g. mode of transmission and dispersal capacity) and host ecology. Where some degree of host-specificity is demonstrated, co-phylogenetic patterns can be used to quantify the frequency of spillover events within and between hosts, and subsequently allow for the investigation of factors associated with increased spillover rate, for example viral/host traits or interface.

In a previous study [18] we reported the discovery of 13 distinct CoVs in bats from Mexico, (9 α-CoVs and 4 β-CoVs) from a survey of 606 bats from 42 different species. This report suggested a level of host-specificity (or host association) for these viruses based on the observed phylogenetic relationships in the RNA dependent RNA polymerase (RdRp). Here we test the hypothesis that CoVs in New World bats have a high degree of host specificity.

Materials and Methods

Sequence data

The CoV sequences (n=32) included in our analyses were described previously [18]. These sequences correspond to nine different genotypes in the α-CoV genus, and four different genotypes in the β-CoV genus (Table S1). The host dataset include mitochondrial DNA Cytochrome-b (Cyt-b) gene sequences from 40 of the 42 bat species sampled in our previous study (Table S2). Cyt-b gene sequences were obtained from GenBank (Table S3). We selected Cyt-b because of its demonstrated reliability in reconstructing the phylogenetic history of Chiroptera [19]. For any bat species that did not have a Cyt-b sequence available in genbank (n=3), we substituted the sequence for the most closely related species. The three substitutions were: *Phyllostomus hastatus* for *Phyllostomus discolor*, *Choeroniscus minor* for *Choeroniscus godmani*, and *Lasiurus borealis* for *Lasiurus intermedius*. Two *Leptonycteris* bat species were not included because sequences from this or a related species were not available. All samples from

these five species were negative for CoV (Table S2).

Phylogenetic analysis

Alignments were constructed using MAFFT [20], executed through Geneious Pro (6.1.6), and manually refined. Avian infectious bronchitis virus (IBV; accession NC001451) was used as the out-group for CoV phylogenies. *Pteropus giganteus* (KJ532397), *Homo sapiens (*NC012920) and *Ornithorhynchus anatinus (*HQ379861) were used for the host phylogenies. Maximum likelihood (ML) trees were generated using RAxML [21] implemented with raxmlGUI [22] under a GTR+gamma substitution model, and bootstrap resampling frequencies were estimated with 1,000 replicates. Bayesian inference (BI) was also used to corroborate the phylogenies generated by ML using MrBayes 3.2.1 executed through Geneious [23] under a GTR substitution model, with 10,000,000 generations, sampling every 5000th generation with 4 heated chains and a burn in length of 1,000,000. ML trees with an identical topology obtained by both ML and BI methods are presented and were used for the co-phylogenetic analysis. Clade support values larger than 50%/0.5 are provided for each phylogram.

Host-Virus Co-Phylogenetic Analysis

Phylogenetic congruence between bats and CoVs was analyzed using topological global co-speciation methods, which are based on statistical tests for congruence between host and virus phylogenies. This approach is considered to be less biased than event-based and cost-based methods where subjective cost schemes are used [24] as it uses an *a posteriori* interpretation that is not integral to the test. We selected global-fit methods, which are able to deal with cases in which multiple viruses are associated with a single host, or where multiple hosts are associated with a single virus.

Host and virus phylogenies were transformed into patristic distance matrices

and a host-virus identity matrix constructed to identify associations. A sum of squared distances gives a value for the overall similarity between trees (ParafitGlobal), which was compared with a distribution of ParafitGlobal values obtained by permutations to assess statistical significance. By removing links one at a time, we assessed the contribution of each individual link to the overall congruence between host-virus phylogenies. Two methods, ParaFit [25] and Procrustean Approach to Cophylogeny (PACo) [26] were used, to test for consistency. Global-fit analyses were performed in ParaFit using package ape [27] implemented in R 3.1.1 [28]. We used 1,000 permutations to implement the global test, and also to test individual CoV-bat links. Each individual CoV-bat interaction is determined to be significant if either ParaFit 1 or Parafit 2 p-values are ≤ 0.05. PACo was also implemented in R using the ape and vegan packages. This test was used to quantitatively assess the congruence between the two phylogenies, as well as the host-virus links contributing to the co-phylogenetic structure. PACo differs from ParaFit by utilizing Procrustean superimposition, in which the viral matrix is rotated and scaled to fit the host matrix. Thus, PACo explicitly tests the dependence of the viral phylogeny upon the host phylogeny, providing a goodness-of-fit statistic (m^2_{XY}) whose significance is established by a randomization procedure [29]. As with ParaFit, PACo requires three matrices, one where host (rows) – virus (columns) associations are indicated as presence/absence (host-virus links), and two genetic distance matrices, which are calculated from the host and viral phylogenetic trees. The host-virus link matrix was transformed into an identity matrix to accommodate multiple associations (e.g. hosts harboring more than one parasite species/lineage and vice versa) and the two distance matrices were transformed into matrices of principal coordinates (PCo).

Results

The host (bat) phylogeny was well resolved and in agreement with previously reported chiropteran phylogenies [19,30,31]. For the viral phylogenies,

the 32 CoV sequences clustered into 13 distinct clades, in either the α-CoV or β-CoV genera, as reported previously [18]. As both ParaFit and PACo allow bats that tested negative for CoV to be included, we were able to use this reasonably resolved phylogeny to obtain a more precise understanding of host-virus interaction. Both ParaFit and PACo analyses provided evidence for a significant congruence between CoV and chiropteran phylogenies (ParaFitGlobal = 48.32226, P≤0.001; m^2_{XY} = 2.016884, P≤0.0001). Twenty-one (66%) of the 32 individual host-virus links were significant based on either the ParaFit1 or Parafit2 values (P≤0.05, Figure 1); results for PACo were consistent with this finding (Figure S1). We considered the potential for differences in the biological and evolutionary histories of α-CoVs and β-CoVs to reflect differences in their co-phylogenetic signals, and so also analyzed host:virus links separately for α-CoVs and β-CoVs. ParaFit and PACo co-speciation signal was detected and the same individual links were significant for both CoV genera (Figure 1). In all cases, we note that the significant congruence observed between both α-CoVs and β-CoVs and their bat hosts appears to be largely driven by CoVs detected in bats from the (related) genera *Artibeus* and *Carollia*.

Mex_CoV-6, 7 and 8 were not shown to be significantly associated with their host species [*Eptesicus fuscus* (P=0.51), *Myotis velifer* (P=0.22), and *Tadarida brasiliensis* (P=0.11)], despite appearing to have some discernible pattern of phylogenetic agreement. However, if host species that were negative for CoV were excluded from the analysis, then Mex_CoV-6, 7 and 8 did become significantly associated with their hosts (P=0.05, P=0.03, P=0.01, respectively). This suggests that the inclusion of host species that appear to be negative for CoVs are influencing the model (which assumes that a negative species is one that does not host any CoV, rather than one in which CoV was simply not detected). Given our data set we cannot be certain that increased sampling wouldn't readily identify additional viruses that, when included in the analysis, would demonstrate a significantly congruent association.

We further conducted analyses at the genus taxonomic level (28 bat genera), where we also found a strong co-speciation signal (ParaFitGlobal = 58.79816, P≤0.001; m^2_{XY} = 2.025327, P≤0.0001), and observed no changes in host-parasite links (same 21/32 significant individual links). Analyses separated for each viral genus showed the same significant pattern of codivergence (for α-CoVs: ParaFitGlobal = 56.37751, P≤0.001; m^2_{XY} = 0.8812547, P≤0.0001, with the same 16/25 significant individual links; and for β -CoVs: ParaFitGlobal = 4.155172, P≤0.001; m^2_{XY} = 0.02741052, P≤0.0001, with the same 5/7 significant individual links, Figure S2).

Discussion

In this study we analyzed the co-phylogenetic patterns of New World bats with both α -CoVs and β -CoVs. We detected congruence in the viral and host phylogenies and use this to suggest that coronaviruses and bats have a history of 'co-speciation' in the New World. Our findings are particularly significant because Mexico represents the transitional zone between Nearctic and Neotropical regions [32] and is considered an evolutionarily 'active' zone where several speciation events have taken place in the past. Indeed, Mexico is recognized as an area of high diversity and endemism - where resident, migratory, endemic, and widespread species coexist [32-34]. It is therefore a unique place to explore co-speciation patterns between the CoVs and their hosts.

Our results indicate that the association of CoVs with bats is strongest at the (host) genus level, as evidence of host-switching was observed between bat species of the same genus [18]. Given the differences in nucleotide substitution rates between viruses and mammalian hosts, we do not assume these viruses have evolved on similar temporal scales, but rather suggest they belong to lineages that have long historical associations with particular groups of bats. We note however that the associations between CoVs and bats from the genera

Artibeus and *Carollia* appear to drive this result, and we therefore suggest that additional CoVs infecting other bat species will be required to fully validate this finding.

A previous study on co-phylogenetic associations failed to identify congruence between CoVs and their bat hosts [35]. This study included CoVs detected in vespertillionid and rhinolophid bats found in China, and showed that these viruses were able to move between different hosts fairly readily. The authors suggested this could be due to biological (virus and/or host) or behavioral (ecological) traits, which would agree with previous suggestions that the large genome size, frequent recombination, and high mutation rate of CoVs could support the ability of CoVs to switch hosts frequently [36]. Indeed, there are certainly several “promiscuous” viruses distributed through the CoV phylogeny [37] including both SARS-related and MERS-related CoVs [4,37-39].

There are also examples however of CoVs that seem to have a fairly restricted host-range [37] suggesting there may be variation in the patterns of host-association. Most of the CoVs detected in the Neotropical ecozone [18,40], including those considered in this study, appear to be restricted to single host genera (based on the limited sampling and discovery efforts conducted to date). If supported over time, this finding suggests that the evolutionary history of CoVs in the New World could be dominated by traits (e.g. immunologic response of hosts to different CoVs) that are conserved at low taxonomic levels (i.e. genus), as there are no apparent ecological barriers that would explain the host-specificity.

Regional variations in the level of virus host-specificity might well support the hypothesis that there are regional differences in the likelihood of a spillover event. If true, hotspot models that seek to identify high-risk areas for disease emergence [6] could be greatly enhanced by a better understanding of these regional virus:host dynamics. Additional surveillance and discovery will be

required to assess whether the CoVs found in Mexico are indeed more host-restricted than CoVs found in other parts of the world.

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References

1. Lau SK, Woo PC, Li KS, Huang Y, Tsoi HW, et al. (2005) Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. Proc Natl Acad Sci U S A 102: 14040-14045.

2. Drexler JF, Corman VM, Muller MA, Maganga GD, Vallo P, et al. (2012) Bats host major mammalian paramyxoviruses. Nat Commun 3: 796.

3. Olival KJ, Islam A, Yu M, Anthony SJ, Epstein JH, et al. (2013) Ebola virus antibodies in fruit bats, bangladesh. Emerg Infect Dis 19: 270-273.

4. Memish ZA, Mishra N, Olival KJ, Fagbo SF, Kapoor V, et al. (2013) Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. Emerg Infect Dis 19: 1819-1823.

5. Quan PL, Firth C, Conte JM, Williams SH, Zambrana-Torrelio CM, et al. (2013) Bats are a major natural reservoir for hepaciviruses and pegiviruses. Proc Natl Acad Sci U S A 110: 8194-8199.

6. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, et al. (2008) Global trends in emerging infectious diseases. Nature 451: 990-993.

7. Wood JL, Leach M, Waldman L, Macgregor H, Fooks AR, et al. (2012) A framework for the study of zoonotic disease emergence and its drivers: spillover of bat pathogens as a case study. Philos Trans R Soc Lond B Biol Sci 367: 2881-2892.

8. Levinson J, Bogich TL, Olival KJ, Epstein JH, Johnson CK, et al. (2013) Targeting surveillance for zoonotic virus discovery. Emerg Infect Dis 19: 743-747.
9. Daszak P, Zambrana-Torrelio C, Bogich TL, Fernandez M, Epstein JH, et al. (2013) Interdisciplinary approaches to understanding disease emergence: the past, present, and future drivers of Nipah virus emergence. Proc Natl Acad Sci U S A 110 Suppl 1: 3681-3688.
10. Switzer WM, Salemi M, Shanmugam V, Gao F, Cong ME, et al. (2005) Ancient co-speciation of simian foamy viruses and primates. Nature 434: 376-380.
11. Perez-Losada M, Christensen RG, McClellan DA, Adams BJ, Viscidi RP, et al. (2006) Comparing phylogenetic codivergence between polyomaviruses and their hosts. J Virol 80: 5663-5669.
12. Ramsden C, Holmes EC, Charleston MA (2009) Hantavirus evolution in relation to its rodent and insectivore hosts: no evidence for codivergence. Mol Biol Evol 26: 143-153.
13. Shah SD, Doorbar J, Goldstein RA (2010) Analysis of host-parasite incongruence in papillomavirus evolution using importance sampling. Mol Biol Evol 27: 1301-1314.
14. Irwin NR, Bayerlova M, Missa O, Martinkova N (2012) Complex patterns of host switching in New World arenaviruses. Mol Ecol 21: 4137-4150.
15. Bennett SN, Gu SH, Kang HJ, Arai S, Yanagihara R (2014) Reconstructing the evolutionary origins and phylogeography of hantaviruses. Trends Microbiol 22: 473-482.
16. Bruyndonckx N, Dubey S, Ruedi M, Christe P (2009) Molecular cophylogenetic relationships between European bats and their ectoparasitic mites (Acari, Spinturnicidae). Mol Phylogenet Evol 51: 227-237.
17. Lei BR, Olival KJ (2014) Contrasting patterns in mammal-bacteria coevolution: bartonella and leptospira in bats and rodents. PLoS Negl Trop Dis 8: e2738.
18. Anthony SJ, Ojeda-Flores R, Rico-Chavez O, Navarrete-Macias I, Zambrana-Torrelio CM, et al. (2013) Coronaviruses in bats from Mexico. J Gen Virol 94: 1028-1038.

19. Agnarsson I, Zambrana-Torrelio CM, Flores-Saldana NP, May-Collado LJ (2011) A time-calibrated species-level phylogeny of bats (Chiroptera, Mammalia). PLoS Curr 3: RRN1212.

20. Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30: 3059-3066.

21. Stamatakis A, Ludwig T, Meier H (2005) RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. Bioinformatics 21: 456-463.

22. Silvestro D, Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. Organisms Diversity & Evolution 12: 335-337.

23. Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574.

24. de Vienne DM, Refregier G, Lopez-Villavicencio M, Tellier A, Hood ME, et al. (2013) Cospeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. New Phytol 198: 347-385.

25. Legendre P, Desdevises Y, Bazin E (2002) A statistical test for host-parasite coevolution. Syst Biol 51: 217-234.

26. Balbuena JA, Miguez-Lozano R, Blasco-Costa I (2013) PACo: a novel procrustes application to cophylogenetic analysis. PLoS One 8: e61048.

27. Popescu AA, Huber KT, Paradis E (2012) ape 3.0: New tools for distance-based phylogenetics and evolutionary analysis in R. Bioinformatics 28: 1536-1537.

28. Team RC (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing: R Foundation for Statistical Computing, Vienna, Austria.

29. Santiago-Alarcon D, Rodriguez-Ferraro A, Parker PG, Ricklefs RE (2014) Different meal, same flavor: cospeciation and host switching of haemosporidian parasites in some non-passerine birds. Parasit Vectors 7: 286.

30. Teeling EC, Springer MS, Madsen O, Bates P, O'Brien S J, et al. (2005) A molecular phylogeny for bats illuminates biogeography and the fossil record. Science 307: 580-584.
31. Bininda-Emonds OR, Cardillo M, Jones KE, MacPhee RD, Beck RM, et al. (2007) The delayed rise of present-day mammals. Nature 446: 507-512.
32. Ortega J, Arita HT (1998) Neotropical-Nearctic limits in Middle America as determined by distributions of bats. Journal of Mammalogy 79: 772-783.
33. Escalante T, Rodríguez G, J. MJ (2004) The diversification of Nearctic mammals in the Mexican transition zone. Biological Journal of the Linnean Society 83: 327-339.
34. Marshall CJ, Liebherr JK (2000) Cladistic Biogeography of the Mexican Transition Zone. Journal of Biogeography 27: 203-216.
35. Cui J, Han N, Streicker D, Li G, Tang X, et al. (2007) Evolutionary relationships between bat coronaviruses and their hosts. Emerg Infect Dis 13: 1526-1532.
36. Woo PC, Lau SK, Huang Y, Yuen KY (2009) Coronavirus diversity, phylogeny and interspecies jumping. Exp Biol Med (Maywood) 234: 1117-1127.
37. Drexler JF, Corman VM, Drosten C (2014) Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. Antiviral Res 101: 45-56.
38. Wacharapluesadee S, Sintunawa C, Kaewpom T, Khongnomnan K, Olival KJ, et al. (2013) Group C betacoronavirus in bat guano fertilizer, Thailand. Emerg Infect Dis 19: 1349-1351.
39. Ge XY, Li JL, Yang XL, Chmura AA, Zhu G, et al. (2013) Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature 503: 535-538.
40. Corman VM, Rasche A, Diallo TD, Cottontail VM, Stocker A, et al. (2013) Highly diversified coronaviruses in neotropical bats. J Gen Virol.

Figures Legends

Figure 1. Tanglegram of cophylogenetic relationships between bat species and Coronaviruses.

Maximum likelihood phylogenies for bat hosts (left: blue, Phyllostomidae; green, Mormoopidae; brown, Molossidae; orange, Vespertilionidae; grey, Emballonuridae) and coronaviruses (right: red, α-CoVs; blue, β-CoVs), with bootstrap support values (≥50, ≥0.5; ML and BI, respectively), rooted with outgroups (in magenta). All 32 CoV sequences are presented on the tree with the CoV clade name followed by PMX number that refers to the host identity. Names of CoV-positive bats are shown in green, and negative ones are shown in black. All 32 host-parasite associations are presented in the tanglegram as grey and black connecting lines (solid lines for α-CoVs and dashed lines for β-CoVs). Black lines indicate significant individual cospeciation links between CoVs and their bat hosts as indicated by ParaFit (P≤0.05), while grey lines represent non-significant links. Bar represents genetic distance among lineages.

Supporting Information

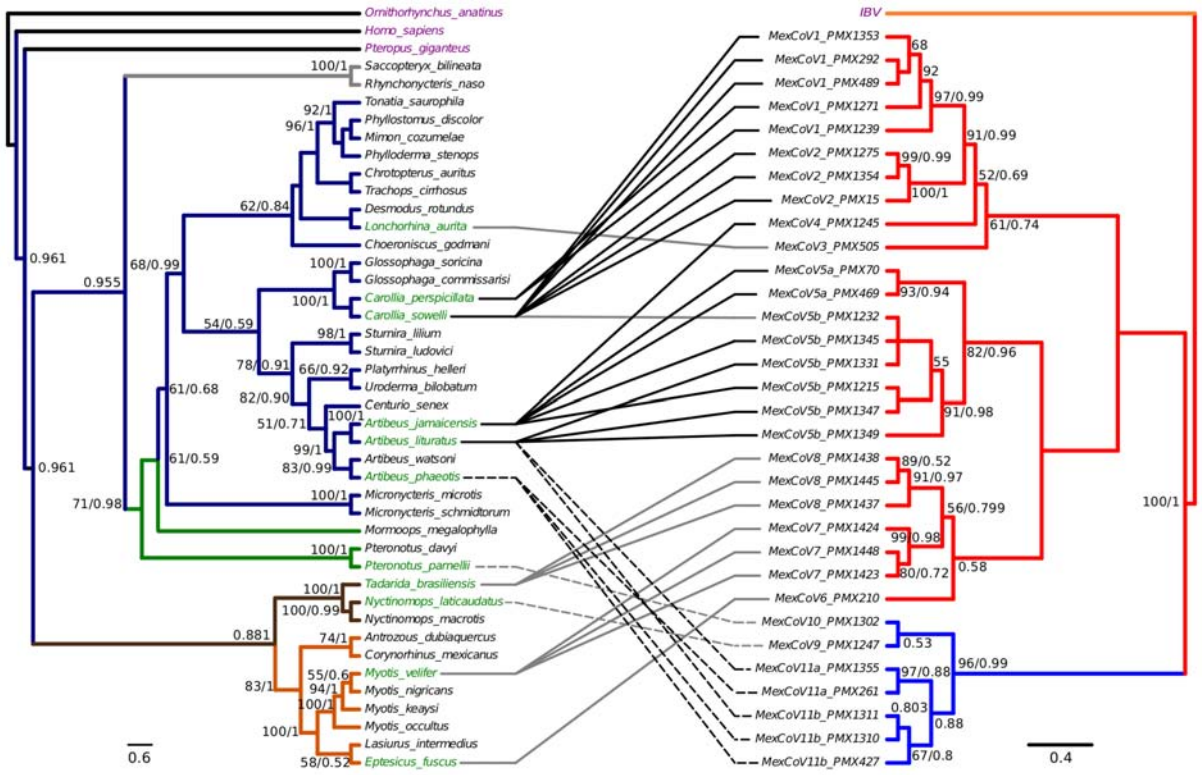
Figure S1. Contributions of individual host-parasite links to the PACo global cospeciation fit.

Figure S2. Tanglegram of cophylogenetic relationships between bat genera and Coronaviruses.

Table S1. Coronavirus sequences detected in Mexican bat hosts.

Table S2. Numbers of all bats captured at each site.

Table S3. Cytochrome b GenBank accession numbers of bat species sampled in Mexico



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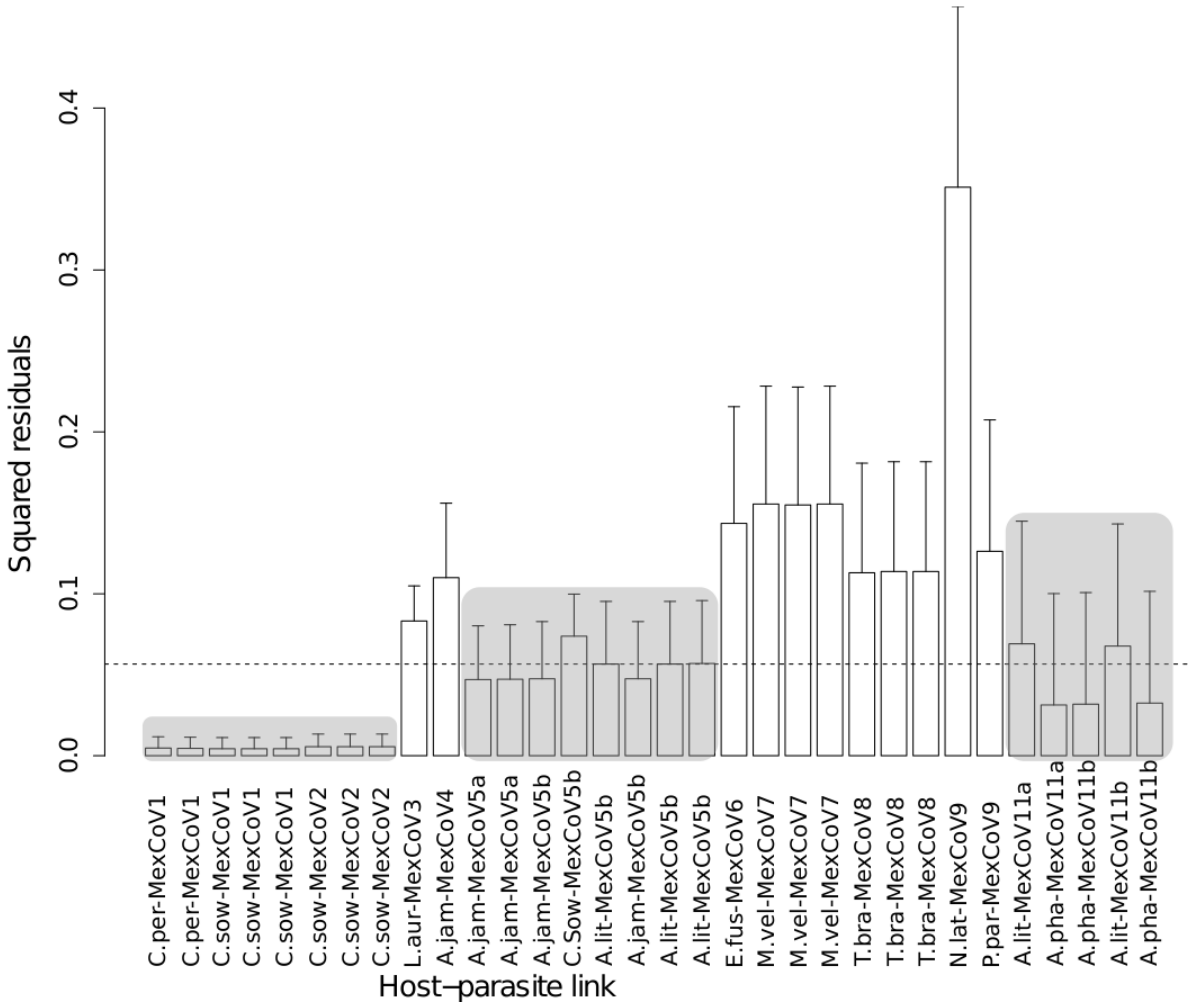

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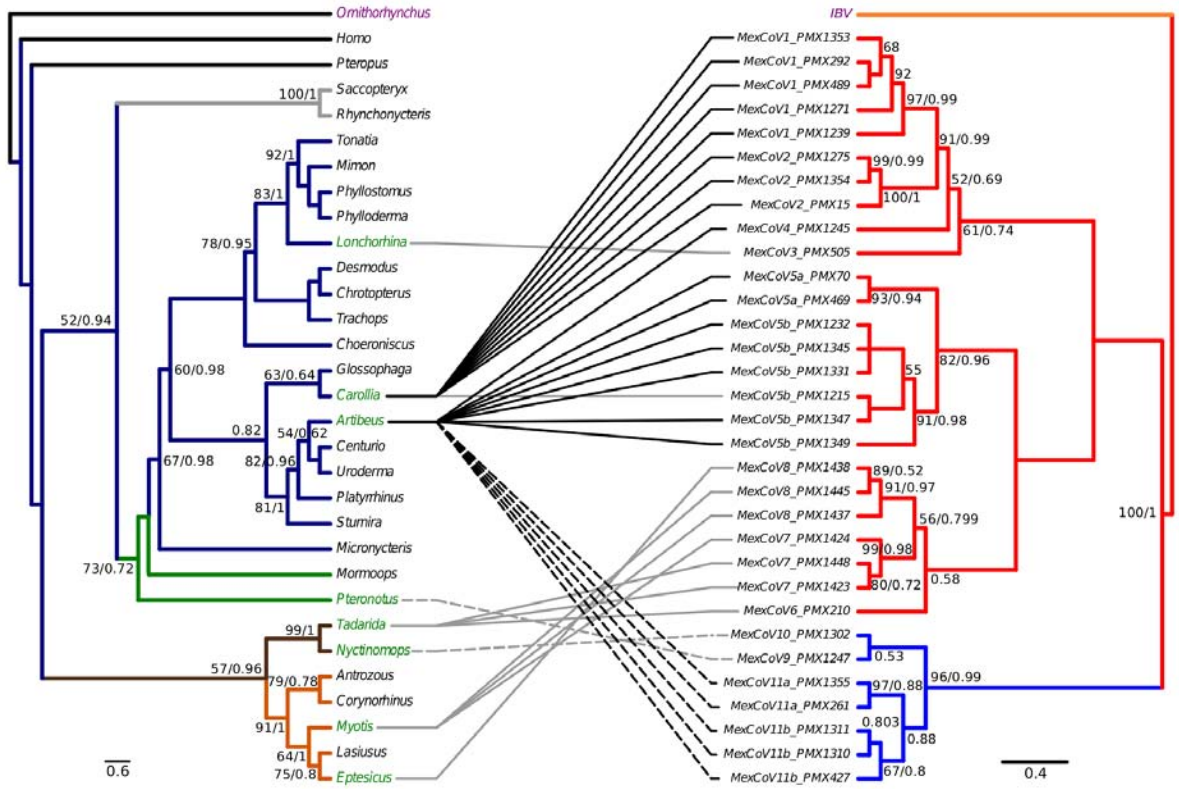

Figure S2. Tanglegram of cophylogenetic relationships between bat genera and Coronaviruses.

Table S1. Coronavirus sequences detected in Mexican bat hosts

GenBank Accession	Host Species	Genotype_ Animal Id	Region
Alphacoronavirus			
KC117186	*Carollia sowelli*	Mex_CoV-1_PMX292	Chiapas
KC117189	*Carollia sowelli*	Mex_CoV-1_PMX489	Chiapas
KC117205	*Carollia sowelli*	Mex_CoV-1_PMX1353	Campeche
KC117193	*Carollia perspicillata*	Mex_CoV-1_PMX1239	Campeche
KC117196	*Carollia perspicillata*	Mex_CoV-1_PMX1271	Campeche
KC117206	*Carollia sowelli*	Mex_CoV-2_PMX1354	Campeche
KC117197	*Carollia sowelli*	Mex_CoV-2_PMX1275	Campeche
	Carollia sowelli	Mex_CoV-2_PMX015	Chiapas
KC117190	*Lonchorhina aurita*	Mex_CoV-3_PMX505	Chiapas
KC117194	*Artibeus jamaicensis*	Mex_CoV-4_PMX1245	Campeche
KC117188	*Artibeus jamaicensis*	Mex_CoV-5a_PMX469	Chiapas
	Artibeus jamaicensis	Mex_CoV-5a_PMX070	Chiapas
KC117201	*Artibeus lituratus*	Mex_CoV-5b_PMX1331	Campeche
KC117202	*Artibeus jamaicensis*	Mex_CoV-5b_PMX1345	Campeche
KC117203	*Artibeus lituratus*	Mex_CoV-5b_PMX1347	Campeche
KC117204	*Artibeus lituratus*	Mex_CoV-5b_PMX1349	Campeche
KC117191	*Artibeus jamaicensis*	Mex_CoV-5b_PMX1215	Campeche
KC117192	*Carollia sowelli*	Mex_CoV-5b_PMX1232	Campeche
KC117184	*Eptesicus fuscus*	Mex_CoV-6_PMX210	Chiapas
KC117208	*Myotis velifer*	Mex_CoV-7_PMX1423	DF
KC117209	*Myotis velifer*	Mex_CoV-7_PMX1424	DF
KC117213	*Myotis velifer*	Mex_CoV-7_PMX1448	DF
KC117210	*Tadarida brasiliensis*	Mex_CoV-8_PMX1437	DF
KC117211	*Tadarida brasiliensis*	Mex_CoV-8_PMX1438	DF
KC117212	*Tadarida brasiliensis*	Mex_CoV-8_PMX1445	DF
Betacoronavirus			
KC117195	*Nyctinomops laticaudatus*	Mex_CoV-9_PMX1247	Campeche
KC117198	*Pteronotus parnellii*	Mex_CoV-10_PMX1302	Campeche
KC117185	*Artibeus phaeotis*	Mex_CoV-11a_PMX261	Chiapas
KC117207	*Artibeus lituratus*	Mex_CoV-11a_PMX1355	Campeche
KC117187	*Artibeus phaeotis*	Mex_CoV-11b_PMX427	Chiapas
KC117199	*Artibeus phaeotis*	Mex_CoV-11b_PMX1310	Campeche
KC117200	*Artibeus lituratus*	Mex_CoV-11b_PMX1311	Campeche

Table S2. Numbers of all bats captured at each site

A total of 606 bats were sampled across three sites. The number (#) of CoV positives (Pos) is indicated in parentheses, together with the number of CoV clades in square brackets.

* Bat species not included in the phylogenetic tree

Family	Bat species	Campeche (# CoV Pos) [CoV clade(s)]	Chiapas (# CoV Pos) [CoV clade(s)]	DF (# CoV Pos) [CoV clade(s)]
Phyllostomidae	*Artibeus jamaicensis*	56 (3) [4, 5b]	37 (2) [5a]	
	Artibeus lituratus	53 (5) [5b, 11a, 11b]	54	
	Artibeus phaeotis	24 (1) [11b]	12 (2) [11a, 11b]	
	Artibeus watsoni	8	9	
	Carollia perspicillata	12 (2) [1]	9	
	Carollia sowelli	41 (4) [1, 2, 5b]	31 (3) [1, 5a]	
	Centurio senex	4		
	*Choeroniscus godmani**		6	
	Chrotopterus auritus		2	
	Desmodus rotundus		7	
	Glossophaga commissarisi		3	
	Glossophaga soricina	9	39	
	*Leptonycteris nivalis**			2
	*Leptonycteris yerbabuenae**			1
	Lonchorhina aurita	1 (1) [3]		
	Micronycteris microtis		1	
	Micronycteris schmidtorum		5	
	Mimon cozumelae		1	
	Phylloderma stenops		2	
	*Phyllostomus discolor**		2	
	Platyrrhinus helleri		6	
	Sturnira lilium	12	22	
	Sturnira ludovici		23	
	Tonatia saurophila		1	

	Trachops cirrhosus		2	
	Uroderma bilobatum	1	4	
Vespertilionidae	*Antrozous dubiaquercus*		18	
	Eptesicus fuscus		1 (1) [6]	
	Corynorhinus mexicanus			1
	*Lasiurus intermedius**	1		
	Myotis keaysi		2	
	Myotis nigricans		1	
	Myotis occultus			1
	Myotis velifer			7 (3) [7]
Molossidae	*Nyctinomops laticaudatus*	5 (1) [9]		
	Nyctinomops macrotis			10
	Tadarida brasiliensis			10 (3) [8]
Mormoopidae	*Mormoops megalophylla*	9	2	
	Pteronotus davyi	1	1	
	Pteronotus parnellii	2 (1) [10]	17	
Emballonuridae	*Rhynchonycteris naso*		1	
	Saccopteryx bilineata		1	
Total bats	42	240	332	34

Table S3. Cytochrome b GenBank accession numbers of bat species sampled in Mexico

* Bat species substituted in the phylogenetic tree

Bat species	GenBank Accession	Positive for CoV	Negative for CoV
Antrozous dubiaquercus	EF222381		x
Artibeus jamaicensis	NC002009	x	
Artibeus lituratus	NC016871	x	
Artibeus phaeotis	DQ869387	x	
Artibeus watsoni	U66516		x
Carollia perspicillata	NC022422	x	
Carollia sowelli	AF511973	x	
Centurio senex	AY604441		x
*Choeroniscus minor**	KC783055		x
Chrotopterus auritus	FJ155481		x
Corynorhinus mexicanus	AY776067		x
Desmodus rotundus	DQ077398		x
Eptesicus fuscus	AF376835	x	
Glossophaga commissarisi	AF382886		x
Glossophaga soricina	AF382867		x
*Lasiurus borealis**	JN209842		x
Lonchorhina aurita	FJ155494	x	
Micronycteris microtis	AY380755		x
Micronycteris schmidtorum	AY380753		x
Mimon cozumelae	FJ155478		x
Mormoops megalophylla	AF338687		x
Myotis keaysi	JX130525		x
Myotis nigricans	AF376864		x
Myotis occultus	AF294500		x
Myotis velifer	JX130589	x	
Nyctinomops laticaudatus	L19729	x	
Nyctinomops macrotis	GQ424045		x
Phylloderma stenops	FJ155480		x
*Phyllostomus hastatus**	FJ155479		x
Platyrrhinus helleri	KJ576924		x
Pteronotus davyi	AF338672		
Pteronotus parnellii	AY604456	x	
Rhynchonycteris naso	EF584188		x
Saccopteryx bilienata	EF584201		x

Sturnira lilium	KC753846		x
Sturnira ludovici	KC753806		x
Tadarida brasiliensis	JF489129	x	
Tonatia saurophila	FJ155487		x
Trachops cirrhosus	FJ155483		x
Uroderma bilobatum	AY169900		x

Viral diversity of bat communities in human-dominated landscapes in Mexico

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Abstract

Using integrative epidemiologic techniques, we studied the changing relationships (beta and phylogenetic beta diversity) of multihost systems and virus associations in bat communities in fragmented landscapes from Chiapas, Campeche and Greater Mexico City. We combined computing applications, molecular detection, and nucleotide sequencing of coronaviruses, hantaviruses, paramyxoviruses and pegiviruses with ecological and phylogenetic analyses. A total of 22 viruses were discovered in 1,067 samples from 42 bat species, representing an estimated 78% of all viral richness in the system. Based on 17 virus genotypes discovered with an equal sampling effort, a total viral richness of 23 genotypes was estimated using a Chao2 statistic model. Using a residual model we categorized host species and habitat types that are prone to harbor higher viral richness. A positive relationship between phylogenetic host diversity with viral diversity ($r = 0.41$, $p < 0.05$) and viral richness ($r = 0.51$, $p < 0.05$) was found. Beta diversity (the rate of change) of viral communities was explained by host beta diversity ($r = 0.86$, $p < 0.05$). To understand the change in viral and host communities we partitioned beta diversity in nestedness (species loss) and turnover (compositional dissimilarity) components. In Chiapas the host beta diversity was explained by nestedness of species composition while phylogenetic host diversity was explained by turnover of the host lineages. Campeche showed a high phylogenetic host nestedness and low host turnover. Beta-diversity and beta-phylogenetic diversity indicated that patterns of local species assemblages and regional abiotic features in human dominated landscapes are significant drivers of viral community composition. Our study represents the first effort in Mexico to study the relationship between viral diversity in bat communities in modified landscapes to understand hosts-viruses relationships.

Keywords: *Disease Ecology*, *Chiroptera, viral richness, alpha diversity, beta diversity, phylogenetic diversity, habitat loss*.

Introduction

Land use change appears to be the primary mechanism driving zoonotic diseases (Patz et al., 2004). The expansion of agricultural production and urbanization have simultaneously modified ecosystem structure and function, community structure (species assemblages), patterns of species distribution and biodiversity (Christian et al., 2009; Gibbs et al., 2009). These modified systems have produced suitable environments for multi-species interactions, particularly hosts, vectors, and/or pathogens (Rubio et al., 2014; Jones et al., 2013; Rivard et al., 2007; McMichael, 2004).

Bats, on one hand, have been considered the likely source of highly pathogenic RNA viruses including lyssaviruses (Banyard et al., 2011), Ebola virus (Leroy et al., 2005), Marburg virus (Towner et al. 2009), Nipah virus (Epstein et al., 2006), Hendra virus (Smith et al., 2011), and coronaviruses (e.g. SARS-coronavirus, MERS-coronavirus) (Hilgenfeld & Peiris, 2013). Bats, on the other hand, are recognized as a key group in the maintenance of ecological systems by providing ecological services such as pollination, seed dispersion and pest control. Additionally, due their response to habitat loss and fragmentation bats are excellent biondicators of environmental changes (Medellín, Equihua, et al., 2008).

To properly understand complex interactions in mutihost systems several ecological and phylogenetic tools have been used. In disease ecology, diversity indices have been used to correlate the number of species (*richness*) and the relative abundance of species present in a given community (*alpha diversity*) with disease prevalence (Suzán et al., 2009) and in microbiomes systems (Olson et al., 2014; Anthony, Epstein, et al., 2013). Diversity indices have also been used to evaluate changes in host-parasite composition in hosts communities at local, regional, and biogeographic scales (Alpha, *beta gamma diversity*) (Scordato & Kardish, 2014; Svensson-Coelho & Ricklefs, 2011). From an evolutionary perspective, host and pathogen phylogenetic relationships have been studied and diversity indices have been incorporated to measure changes in host species

community assemblages through environmental gradients (Helmus et al., 2007; Webb et al., 2002). These phylogenetic methods offer additional dimensions to explore host-parasite interactions in time, such as; host specificity, host-parasite co-evolution, host switching events, and phylogenetic barriers preventing pathogen transmission (Poulin et al., 2011; Streicker et al., 2010; Legendre et al., 2002). The study of ecological and phylogenetic interactions between host-pathogen systems integrates the role of environmental influences on host and pathogen distributions across time and spatial scales and across different levels of biological organization beyond taxonomic levels (Hawley & Altizer, 2011)

In this study we examined the relationship between host diversity and the diversity of four viral taxa in bats from human dominated landscapes in Mexico. Two hypotheses were tested related to the effect of host species and host phylogenetic diversities on viral diversity and the influence of habitat type in the composition of host and viral communities. First, we hypothesized that (1) host communities with high species and phylogenetic diversities will support high values of viral diversity; (2) changes in host and viral community composition across a habitat type will be reflected in high values of beta diversity and phylogenetic-beta diversity.

Material and Methods

Sample collection

Bats were captured at three different sites in Mexico at Reserva de la Biosfera Montes Azules (RBMA) in Chiapas, the Reserva de la Biosfera Calakmul (RBC) in Campeche, and Greater Mexico City (GMC) that include Distrito Federal and Metropolitan Area. The first two sites, located in southeastern Mexico, represent regions of high species diversity and are characterized by large tracts of continuous primary vegetation, while the Greater Mexico City site is highly urbanized with vegetation patches. A high evergreen forest characterizes RBMA while RBC is dominated by tropical semi-deciduous forest; both regions have high anthropogenic

pressure. In RBMA and RBC bats were collected from three different habitat types: *'Forested'* (Fd), where signs of human impact are largely absent and original vegetation persists, *'Fragmented'* (F), areas of primary vegetation are interspersed with agricultural/rangeland and *'Disturbed'* (D), the transition zone between areas of secondary vegetation and agricultural/rangeland and by the presence of urban areas. In the Greater Mexico City sites, bats were captured in '*Urban'* (U), human dominated areas and *'Fragmented'* habitats (F). We used 5 mist-nets (each 9 x 3 m wide) and were opened at dusk and remained open four consecutive hours. Each habitat was sampled once in six months. Identification of bats was made using a field guide (Medellín, Arita, et al., 2008). The minimal distance in RBMA was 2 km while in RBC was 10 km. A mantel test was performed to ensure sites independence due to the geographic distance (RBMA; r = 0.55, p =0.01; RBC r = 0.57 p = 0.006). Oral and rectal swabs, and when possible blood samples were collected from each animal. Samples were collected in a lysis buffer and preserved at -80°C until transfer to the Center for Infection and Immunity, Columbia University, New York for viral screening.

Virus discovery

A total of 1,067 samples from 608 individuals representing 42 bat species were tested for the five viral families/genera (Table S1). Total nucleic acid was extracted from all samples using the EasyMag® (bioMérieux, Inc) platform, and cDNA synthesis performed using SuperScript® III first strand synthesis supermix (Invitrogen), all according the manufacturer's instructions. Viral discovery was performed using broadly reactive consensus PCR primers, targeting the L-Segment for hantavirus detection (Klempa et al., 2006), and the polymerase (pol) gene for paramyxovirus detection (Tong et al., 2008). PCR products of the expected size were cloned into Strataclone™ PCR cloning vector and sequenced using standard M13R primers. Coronavirus, hepacivirus and pegivirus detections have been previously reported and these viral sequences were detected in the same 1,067 samples (Quan et al., 2013; Anthony, Ojeda-Flores, et al., 2013).

Estimates and Completeness of Viral Richness

We evaluated our sampling effort (the number of samples tested for a given virus), using two methods: by producing rarefaction and extrapolation curves and by calculating the values of the residuals of the linear regression between viral richness within a host and sampling effort by host. Rarefaction and extrapolation curves are statistical techniques to estimate the number of species for a given number of samples (Chao & Jost, 2012; Magurran, 2004) allowing the evaluation of the sampling effort and estimate the number of host samples required to obtain a viral richness value with 95% of confidence (Chao et al., 2014). We evaluated the viral richness, defined as unique viruses discovered in the 1,067 samples, by constructing a sample size based rarefaction and extrapolation curves using a three-fold original sample effort (3,201 samples) (Chao et al., 2014) with the R iNEXT library (Hsieh et al., 2013). The same methodology was conducted to explore viral completeness by habitat type. For this we only considered samples with the same number of PCR screenings (coranaviruses, paramyxoviruses and hantaviruses). In order to identify host species associated with higher viral richness we used a methodology proposed by Herbreteau (2012). We calculated residual values from the linear regression of the logarithm of viral richness and the logarithm of sampling effort for each species and in each disturbance level. These data were logarithmically transformed to stabilize the variance. Host species or disturbance levels with positive or negative residual values were identified as host species with more or less viral richness than expected by the regression model (Herbreteau et al., 2012).

Host and Viral Diversity

In order to study regional host and viral alpha diversities, abundance matrixes (host and virus) were constructed, where rows were disturbance level and columns were (i) host species and (ii) viruses discovered in each disturbance level. Using the R vegan library (Oksanen et al., 2013), a Shannon-Wiener diversity

index (Shannon, 1948) was calculated for each matrix. Values ranged from 0 when there is only one species present to 1 when all species are equally represented in the sample (Magurran, 2004).

Phylogenetic Diversity and Host Specificity

The mammalian super tree (Bininda-Emonds et al., 2007) was used to calculate phylogenetic diversity (PD) of host communities using the R Picante library (Kembel et al., 2010). The PD was measured by calculating the sum of the total branch length of the host species phylogeny sampled in each habitat type (Faith, 1992). Because data of host taxonomic diversity were not normally distributed only phylogenetic analyses were performed. The relationship between viral richness and viral diversity with host PD was explored using a linear model. To quantify host-viral taxonomic associations we used a modified index of host specificity proposed by Poulin and Mouillot, 2003, that measures the PD of host communities associated to each virus. Viruses with high values of host specificity indicates that they have a plasticity to infect a wide range of host, while viruses with lower values are restricted to a few closely related host species (Poulin et al., 2011; Poulin & Mouillot, 2003)

Beta Diversity and Phylogenetic Beta Diversity

A Pearson correlation test was performed to explore the relationship between host BD and PBD with the change of composition of viral communities by habitat type calculated with Sorensen index. To evaluate the change in composition of viral and host communities (beta-diversity; BD) within regions we used measures of beta-diversity: spatial turnover (β_{SIM}) and nestedness (β_{SNE}) components (Baselga, 2010). Spatial turnover (β_{SIM}) measures the replacement of species by another species due to environmental factors or spatial isolation, such as by habitat fragmentation (Calderón-Patrón et al., 2012). The nestedness component (β_{SNE}) measures if sites with smaller number of species are subset of

richer sites (Ulrich et al., 2009). These components were calculated for taxonomic and phylogenetic beta-diversity analyses. The phylogenetic beta-diversity (PBD) measures how phylogenetic relatedness changes across space in the same manner that BD measures how species composition changes across space (Graham & Fine, 2008). The PBD between disturbance levels was obtained using the inverse of the PhyloSor index (Bryant et al., 2008). This index represents shared branches between communities from two sites. Values range from 0 when no species are shared to 1 when all species between locations are the same. The methodology of Leprieur (2012) was used to calculate the phylogenetic turnover ($P\beta_{SIM}$) and to measure the phylogenetic dissimilarity, nested patterns of species assemblages ($P\beta_{SNE}$). The functions *beta.multi* for P and *phylo.beta.multi* for PB from the R betapart library were also applied to calculate the influence of each component on host and viral community composition by habitat type in each region (Baselga & Orme, 2013).

Results

Viral Community Data

A total of 4,139 consensus PCR assays were performed for viral detection, including coronaviruses (CoV´s, n = 1,067), paramyxoviruses (PMV´s, n = 1,067), hantaviruses (HTV´s, n = 1,067), pegiviruses (PGV´s, n = 469) and hepacivirus (HPV´s, n = 469). A viral richness (S) of 22 virus genotypes in 46 positive samples from a total of 1,067 samples, 13 for coronavirus, 2 for paramyxovirus, 2 for hantavirus, and 5 for pegivirus were obtained (Table S2). No hepacivirus and no co-infections were detected. This viral richness is associated with 17 bat species from 12 genera and 4 families (Figure 1A). In forested habitat a total of 11 viruses were detected followed by fragmented habitat (10) disturbed (8) and urban (3) (Figure 1B). The bat species harboring viral richness greater than one were all phyllostomid bats: *Carollia sowelli* (S = 5), *Artibeus lituratus* (S = 4), *Artibeus*

jamaicensis (S = 3), *Artibeus phaeotis* (S = 3) and *Trachops cirrhosus* (S = 2) (Figure S1).

Estimates and Completeness of Viral Richness

Based on the 17 virus genotypes discovered, we estimated a maximum richness of 23 genotypes using a Chao2 statistic model (Chao & Jost, 2012). The sampling effort of 1,067 samples represents a completeness of 81% in relation to the estimated viral richness. The rarefaction sample coverage function estimates a 97% of completeness with a sample size of 3,201 (three-fold sample size) (Figure 2). The comparison between habitats showed the highest value of completeness (53%) in forested habitat, followed by disturbed (43%) and fragmented (15%). The estimates of viral richness with a three-fold original sample effort by habitat were 24 from fragmented habitat, 19 for forested and 10 for disturbed (Table 1).

Positive relationships were observed between sampling effort and host viral richness ($R^2 = 0.44$, $p < 0.01$) and between sampling effort and habitat type viral richness ($R^2 = 0.37$ $p < 0.05$). *Sturnira lilium*, *Pteronotus parnelli* and *Artibeus jamaicensis* were associated with more viral richness than expected by linear model between host viral richness and sampling effort whereas *Trachops cirrhosus*, *Lonchorhina aurita* and *Eptesicus fuscus* (Figure 3.A), were recognized as host species associated with lower viral richness than expected. RBMA fragmented habitat type harbor the highest number of viruses than expected while RBC fragmented habitat type was identified as the site with smallest viral richness than expected by the model (Figure 3.B).

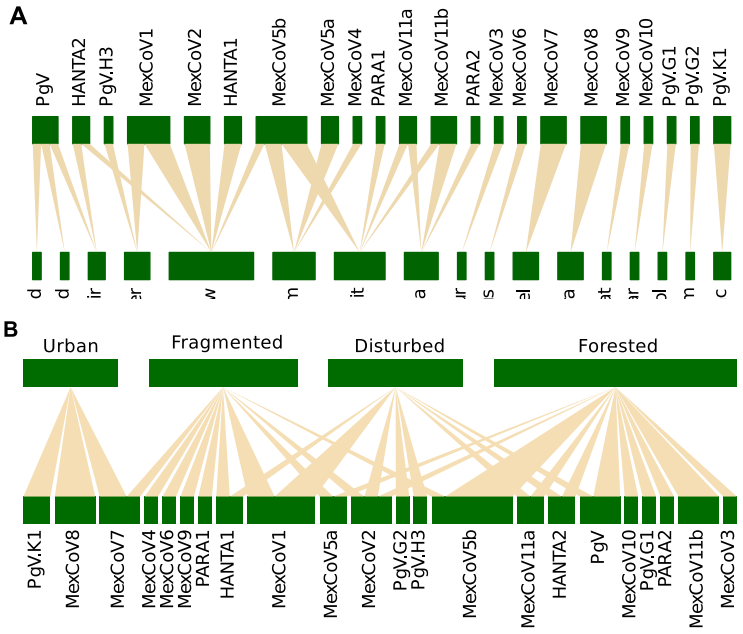

Figure 1. Bipartite graph of 22 virus genotypes discovered in 17 bat species. A. Viral richness associated to bats. The width of the green boxes represents viral and positive hosts abundances. B. Viral richness associated to habitat type.

Table 1. Estimates of viral richness and samples completeness by habitat type. The asterisk indicates real data.

Habitat	Sample Size	Richness	Sampled Completness
Total	1067*	17*	81%
	1677	18	95%
	3201	23	97%
Forested	405*	9*	53%
	1215	19	76%
	3201	27	95%
Fragmented	217*	10*	15%
	651	24	40%
	3201	48	94%
Disturbed	395*	5*	43%
	1185	10	97%
	3201	11	99%
Urban	49*	2*	100%

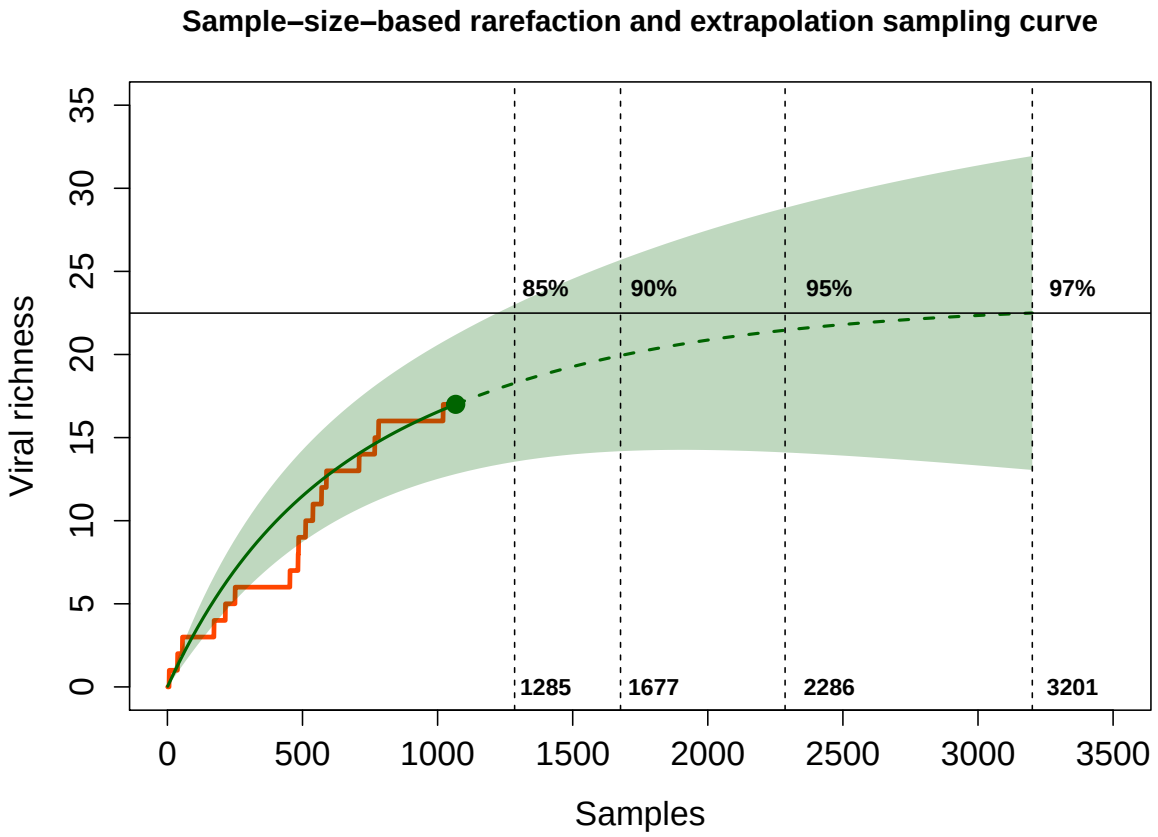

Figure 2. Rarefaction and extrapolation sampling curve based on 1,067 samples. Orange line represents accumulation curve of virus genotypes over samples tested. Green point, viral richness = 22 in 1,067 samples analyzed for virus tested. Solid green line: rarefaction curve, green dashed line: extrapolation-sampling curve. The numbers of samples needed to obtain the percentage of completeness of 85, 90, 95 and 97% are presented.

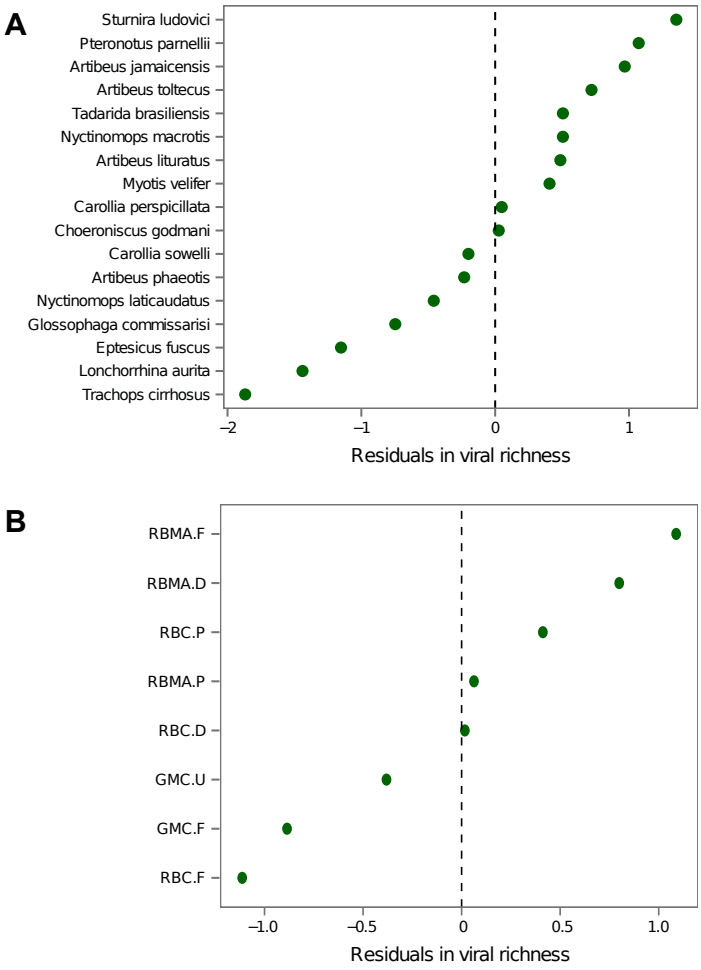

Figure 3. Distribution of residuals values from the linear relation (A) between host viral richness and sampling effort and (B) between habitat type viral richness and sampling effort. Host species and habitats type were reordered by residuals of the sampling effort regression.

Host and Viral Diversity

Montes Azules was the region with more virus genotypes discovered (12), followed by Calakmul (11) and Greater Mexico City, respectively (3) (Figure 4). As we expected, RBMA was the most diverse region in terms of host species and host phylogeny, being C the most diverse area in both diversity scales (H = 2.79, PD = 484.8). Interestingly, D presented high values of both, species and phylogenetic

diversities compared to F (Table 2). A difference in host species diversity ($F = 13.63$, $df = 2$, $p < 0.01$) and host phylogenetic diversity was observed ($F = 16.71$, $df = 2$, $p < 0.01$) at regional scale contrary to viral diversity comparison ($F = 3.73$, $df = 2$, $p > 0.05$). A significant relationship between viral richness and viral diversity with, host phylogenetic diversity was observed. A total of 41% ($p < 0.05$) and 51% ($p < 0.05$) of viral richness and viral diversity variance was explained by phylogenetic diversity, respectively. This suggests that host community composition at each habitat type is determining both, viral richness and viral diversity.

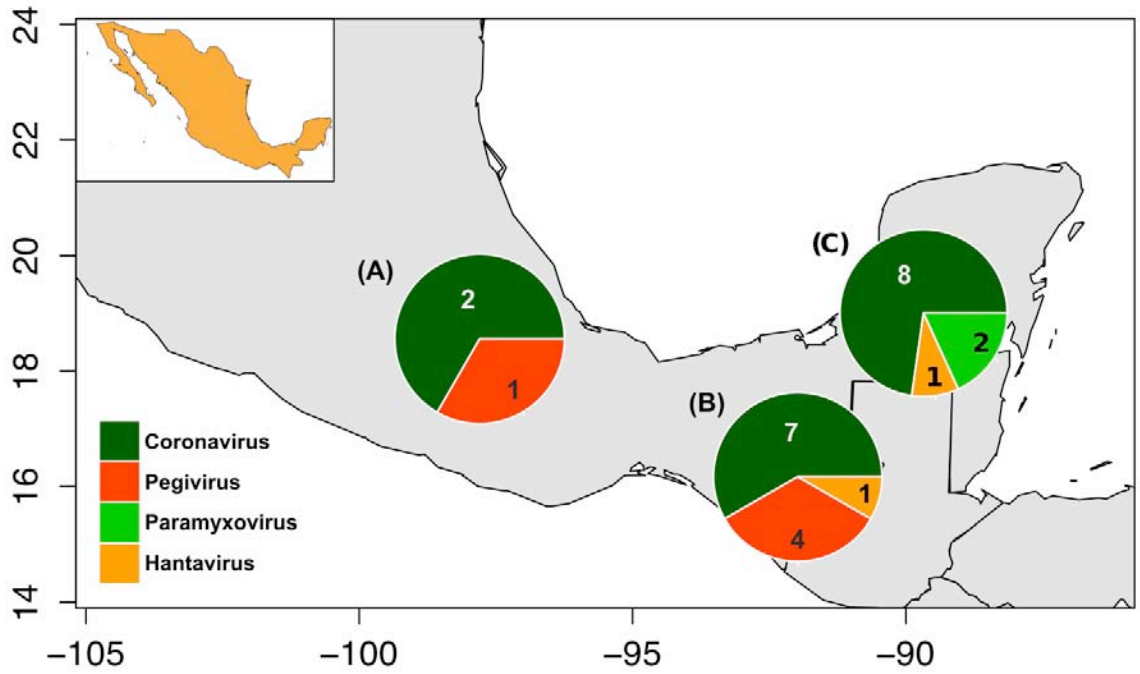

Figure 4. Viral richness in the three regions of study: (A) Greater Mexico City, (B) Montes Azules and (C) Calakmul. Number of virus genotypes discovered per region is shown.

Table 2. Host and viral diversities per habitat type for the 3 regions. Richness (S), Shannon – Weiner diversity index (H), phylogenetic diversity (PD).

		Host			Virus	
Region	**Habitat**	**S**	**H**	**PD**	**S**	**H**
Montes Azules	Forested	26	2.79	484.8	8	2.043
	Fragmented	14	2.207	332.8	2	0.693
	Disturbed	20	2.622	418.2	5	1.56
Calakmul	Forested	12	1.906	260.1	4	1.906
	Fragmented	8	1.853	208.8	7	1.853
	Disturbed	13	1.986	289.1	4	1.986
Greater Mexico City	Fragmented	1	0	60	1	0
	Urban	4	1.155	137.6	3	1.079

Phylogenetic Host Specificity

From 22 viruses discovered, only six were associated to more than two host species and 16 viruses were detected in one host species (Table S3). The flavivirus PgV was the virus with the lowest value of phylogenetic host specificity (114.5) and was detected in three species from three different phyllostomidae subfamilies; Stenodermatinae and phyllostominae, both from forested sites and glossophaginae from disturbed habitat. Hanta 2 (87.8) were detected in two bat species from different habitats; *Carollia sowellii* (disturbed) and *Trachops cirrhosus* (forested), while the coronavirus MexCoV 5b was found in three bat species, two of which belongs to the same genus; *Artibeus jamaicensis, A. lituratus* (forested) and *C. sowellii* (fragmented). MexCov 11a (68.3), Mex CoV 11b (68.3) and MexCov 1 (65.6) were detected in two bat species from the same genus (Figure 1A).

Beta Diversity

We found that 86% ($p < 0.05$) of the beta diversity of viral communities was explained by the turnover of the host species between habitats. The same trend was observed with host PBD and virus beta diversity however was not statistically significant ($r = 0.79$, $p > 0.05$). For Montes Azules, the community dissimilarity in

host species composition through the habitat gradient was relatively high (β_{SOR} = 0.49), and is explained by the nestedness component (β_{SNE} = 0.57), because most of the bat species sampled in low rich sites are contained in the richest site (forested = 26 species). PBD showed different behavior; although the value of overall PBD is similar ($P\beta_{SOR}$ = 0.42), the phylogenetic composition change was partially explained by the turnover component ($P\beta_{SIM}$ = 0.25), due to the low phylogenetic relationship contained in the species subset replaced by nestedness. The overall beta diversity in RBC was relatively low (β_{SOR} = 0.37), suggesting that the habitat type has a low influence on the species assemblage. The observed pattern in RBC was different to RBMA, while species composition dissimilarity was moderately driven by species replacement ($P\beta_{SIM}$ = 0.22). In contrast to RBMA, the PBD analysis indicated a replacement dominated by high phylogenetically related species ($P\beta_{SNE}$ = 0.79). The interpretation of the pattern observed in GMC is limited, because only two communities were sampled and the nestedness component is impossible to calculate. In RBMA and RBC we observed high overall values of beta diversity in viral communities (Table S3), and both were explained by the turnover component (β_{SIM} =0.66), suggesting that changes in habitat quality drives a high replacement of virus species regardless of host composition.

Discussion

In this study we evaluated the relationship between bat diversity and the diversity of 4 medically important viral families within an environmental gradient in human-dominated landscapes in southern Mexico. Combining computing applications and leading-edge molecular techniques for viral identification with ecological and phylogenetic analyses, we measured the viral community turnover in bat communities. A strong relation between viral richness and viral diversity with host phylogenetic diversity was detected. Generalist species were associated with more viruses than expected and a positive relationship between beta diversity of both, viral and bat communities through the habitat gradient were detected.

As hypothesized, significant positive correlation between phylogenetic diversity with viral richness and viral diversity was detected, supporting on one hand, the habitat heterogeneity hypothesis (Lawton, 1983), that proposes a strong relationship between environmental diversity, in this case, the phylogenetic host diversity with biological diversity (viral diversity), and supporting, on the other hand, the keystone structure hypothesis (Twes 2004) that refers to “Keystone structures” as host species that provide distinct resources, which may be linked to different viral species. Considering bat hosts as habitats, the keystone structures can be categorized in characteristics that relate to reproduction and abundance (reproductive potential, longevity, trophic guild, abundance and adult mass), to transmission potential (home range, diet breath and roost size) and phylogenetic relationships (phylogenetic distance and phylogenetic distinctiveness). Our findings suggest that factors such as fragmentation and habitat loss drive species assemblages resulting in areas of major risk for zoonotic disease emergence, as proposed by (Gay et al., 2014; Kamiya et al., 2014; Rubio et al., 2014). Future studies are required to identify which host traits determine the viral communities assemblages.

The phylogenetic host specificity calculated in this study not only reflects the number of bat species infected by a single virus, but also it helps to explore the phylogenetic relationship among these species. Our findings show that few viral species possess high host plasticity like PgV and Hanta 2, while most viruses detected showed high phylogenetic specificity. Due to the quality of our data, we cannot conclude that viruses found in only one bat species are strictly exclusive to them and further studies are needed. However, the coronavirus family showed a high host phylogenetic specificity at genus level as shown in Anthony, Ojeda-Flores, et al., 2013; Ojeda-Flores et al. *In review*).

Changes in viral communities composition through the evaluated anthropogenic landscapes showed a strong dependence on the host species turnover, however this relationship was not statistically significant when host PBD was considered. In general, different patterns between regions and between

diversity levels were reported. For instance, Montes Azules is characterized by a nestedness process in host communities composition and changes are a reflect of a high PD host pool, while in Calakmul BD values were relatively low due to low PD host diversity, and the PBD was explained by nestedness component. As hypothesized, we found high values of beta diversity in viral communities supporting the hypothesis of perturbation, where land use change modifies parasite dynamics in multihost systems by cross-species shifting in parasite transmission (Murray & Daszak, 2013). It has been widely proposed that habitat transformation drives the exposure of novel hosts to a rich pool of parasites, specially in high diversity regions, influencing the cross-species transmission rate (Brearley et al., 2013; Murray & Daszak, 2013; Lloyd-Smith et al., 2009).

The use of beta analyses at both scales: taxonomic and phylogenetic has provided a useful tool to understand if host species or environmental filters can determine the parasite composition (Scordato & Kardish, 2014; Svensson-Coelho & Ricklefs, 2011). We could not demonstrate that host phylogeny determines the composition of viral communities due to spatial scale limitations in our study. Further studies are necessary to test the correlation between host phylogeographical structure and beta diversity of viral communities within larger spatial scales or in communities separated by geographic barriers (vicariance processes).

Conclusions

Our study represents one of the few that integrates viral and host diversity within environmental gradient in human-dominated landscapes in vertebrates and represents the first study in Mexico measuring viral community turnover in bats. Combining molecular and ecological methodologies and the integration of phylogenetic component in the beta diversity analyses helped to distinguish different patterns of host composition variation through the habitat gradient type.

This effort represents an important tool to monitor potential viral richness in wildlife surveillance systems.

References

Allendorf, S. D., Cortez, A., Heinemann, M. B., Harary, C. M. A., Antunes, J. M. a P., Peres, M. G., et al. (2012). Rabies Virus Distribution in Tissues and Molecular Characterization of Strains from Naturally Infected Non-Hematophagous Bats. *Virus research*, 165(2), 119–25.

Anthony, S. J., Epstein, J. H., Murray, K. A., Navarrete-Macias, I., Zambrana-torrelio, C. M., Solovyov, A., et al. (2013). A Strategy To Estimate Unknown Viral Diversity in Mammals Simon. *MBio*, 4(5).

Anthony, S. J., Ojeda-Flores, R., Rico-Chávez, O., Navarrete-Macias, I., Zambrana-Torrelio, C. M., Rostal, M. K., et al. (2013). Coronaviruses in Bats from Mexico. *The Journal of general virology*, 94(Pt 5), 1028–38.

Banyard, A. C., Hayman, D., Johnson, N., McElhinney, L. & Fooks, A. R. (2011). Bats and Lyssaviruses. *Advances in virus research*, 79, 239–89.

Baselga, A. (2010). Partitioning the Turnover and Nestedness Components of Beta Diversity. *Global Ecology and Biogeography*, 19(1), 134–143.

Baselga, A. & Orme, C. (2013). Betapart: An R Package for the Study of Beta Diversity. *Methods Ecol.Evol.*, 3, 808–812.

Bininda-Emonds, O. R. P., Cardillo, M., Jones, K. E., MacPhee, R. D. E., Beck, R. M. D., Grenyer, R., et al. (2007). The Delayed Rise of Present-Day Mammals. *Nature*, 446(7135), 507–12.

Brearley, G., Rhodes, J., Bradley, A., Baxter, G., Seabrook, L., Lunney, D., et al. (2013). Wildlife Disease Prevalence in Human-Modified Landscapes. *Biological reviews of the Cambridge Philosophical Society*, 88(2), 427–42.

Bryant, J. A., Lamanna, C., Morlon, H., Kerkhoff, A. J., Enquist, B. J. & Green, J. L. (2008). Microbes on Mountainsides: Contrasting Elevational Patterns of

Bacterial and Plant Diversity. *Proceedings of the National Academy of Sciences*, 105, 11505–11511.

Calderón-Patrón, J. M., Moreno, C. E. & Zuria, I. (2012). La Diversidad Beta: Medio Siglo de Avances. *Revista Mexicana de Biodiversidad*, 83(3), 879–891.

Canuti, M., Eis-Huebinger, A. M., Deijs, M., de Vries, M., Drexler, J. F., Oppong, S. K., et al. (2011). Two Novel Parvoviruses in Frugivorous New and Old World Bats. *PloS one*, 6(12), e29140.

Chao, A., Gotelli, N. J., Hsieh, T. C., Sander, E. L., Ma, K. H., Colwell, R. K., et al. (2014). Rarefaction and Extrapolation with Hill Numbers : A Framework for Sampling and Estimation in Species Diversity Studies. *Ecological Monographs*, 84(1), 45–67.

Chao, A. & Jost, L. (2012). Coverage-Based Rarefaction and Extrapolation: Standardizing Samples by Completeness rather than Size. *Ecology*, 93(12), 2533–2547.

Chen, L., Liu, B., Yang, J. & Jin, Q. (2014). DBatVir: The Database of Bat-Associated Viruses. *Database : the journal of biological databases and curation*, 2014, bau021.

Christian, K., Isabelle, L. V., Frédéric, J. & Vincent, D. (2009). More Species, Fewer Specialists: 100 Years of Changes in Community Composition in an Island Biogeographical Study. *Diversity and Distributions*, 15(4), 641–648.

Cleaveland, S., Laurenson, M. K. & Taylor, L. H. (2001). Diseases of Humans and Their Domestic Mammals: Pathogen Characteristics, Host Range and the Risk of Emergence. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 356(1411), 991–9.

Epstein, J. H., Field, H. E., Luby, S., Pulliam, J. R. C. & Daszak, P. (2006). Nipah Virus: Impact, Origins, and Causes of Emergence. *Current Infectious Disease Reports*, 8, 59–65.

Faith, D. P. (1992). Conservation Evaluation and Phylogenetic Diversity. *Biological Conservation*, 61(1), 1–10.

Galindo González, J. (2004). Clasificacion de Los Murciélagos de La Region de Los Tuxtlas, Veracruz, Respecto a Su Respueta a La Fragmentación Del Hábitat. *Acta Zoologica Mexicana*, 20(002), 239–243.

Gay, N., Olival, K. J., Bumrungsri, S., Siriaroonrat, B., Bourgarel, M. & Morand, S. (2014). Parasite and Viral Species Richness of Southeast Asian Bats: Fragmentation of Area Distribution Matters. *International journal for parasitology. Parasites and wildlife*, 3(2), 161–70.

Gibbs, K. E., MacKey, R. L. & Currie, D. J. (2009). Human Land Use, Agriculture, Pesticides and Losses of Imperiled Species. *Diversity and Distributions*, 15, 242–253.

Graham, C. H. & Fine, P. V. a. (2008). Phylogenetic Beta Diversity: Linking Ecological and Evolutionary Processes across Space in Time. *Ecology letters*, 11(12), 1265–77.

Hawley, D. M. & Altizer, S. M. (2011). Disease Ecology Meets Ecological Immunology: Understanding the Links between Organismal Immunity and Infection Dynamics in Natural Populations. *Functional Ecology*, 25(1), 48–60.

Helmus, M. R., Savage, K., Diebel, M. W., Maxted, J. T. & Ives, A. R. (2007). Separating the Determinants of Phylogenetic Community Structure. *Ecology letters*, 10(10), 917–25.

Herbreteau, V., Bordes, F., Jittapalapong, S., Supputamongkol, Y. & Morand, S. (2012). Rodent-Borne Diseases in Thailand: Targeting Rodent Carriers and Risky Habitats. *Infection ecology & epidemiology*, 2.

Hilgenfeld, R. & Peiris, M. (2013). From SARS to MERS: 10 Years of Research on Highly Pathogenic Human Coronaviruses. *Antiviral Research*, 100, 286–295.

Holmes, E. C. & Drummond, A. J. (2007). The Evolutionary Genetics of Viral Emergence. *Current Topics in Microbiology and Immunology*, 315, 51–66.

Hsieh, T. C., Ma, K. H. & Chao, A. (2013). iNEXT: Interpolation and Extrapolation.

Jones, B. A., Grace, D., Kock, R., Alonso, S., Rushton, J., Said, M. Y., et al. (2013). Zoonosis Emergence Linked to Agricultural Intensification and

Environmental Change. *Proceedings of the National Academy of Sciences of the United States of America*, 110(21), 8399–404.

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L., et al. (2008). Global Trends in Emerging Infectious Diseases. *Nature*, 451(7181), 990–3.

Kamiya, T., O'Dwyer, K., Nakagawa, S. & Poulin, R. (2014). Host Diversity Drives Parasite Diversity: Meta-Analytical Insights into Patterns and Causal Mechanisms. *Ecography*, 37(7), 689–697.

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., et al. (2010). Picante: R Tools for Integrating Phylogenies and Ecology. *Bioinfomatics*, 26, 1463–1464.

Klempa, B., Fichet-Calvet, E., Lecompte, E., Auste, B., Aniskin, V., Meisel, H., et al. (2006). Hantavirus in African Wood Mouse, Guinea. *Emerging infectious diseases*, 12(5), 838–40.

Lawton, J. H. (1983). Plant Architecture and the Diversity of Phytophagous Insects. *Annual Review of Entomology*, 28(1), 23–39.

Legendre, P., Desdevises, Y. & Bazin, E. (2002). A Statistical Test for Host-Parasite Coevolution. *Systematic biology*, 51(2), 217–34.

Leroy, E. M., Kumulungui, B., Pourrut, X., Rouquet, P., Hassanin, A., Yaba, P., et al. (2005). Fruit Bats as Reservoirs of Ebola Virus. *Nature*, 438, 575–576.

Lloyd-Smith, J. O., George, D., Pepin, K. M., Pitzer, V. E., Pulliam, J. R. C., Dobson, A. P., et al. (2009). Epidemic Dynamics at the Human-Animal Interface. *Science (New York, N.Y.)*, 326(5958), 1362–7.

Magurran, A. E. (2004). *Measuring Biological Diversity*. Oxford, UK: Blackwell Science Ltd.

McMichael, A. J. (2004). Environmental and Social Influences on Emerging Infectious Diseases: Past, Present and Future. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 359(1447), 1049–58.

Medellín, R., Arita, H. T. & Sánchez, H. O. (2008). *Identificación de Los Murciélagos de México. Clave de Campo*. México D.F.: Instituto de Ecología, UNAM.

Medellín, R., Equihua, M. & Amin, M. (2008). Bat Diversity and Abundance as Indicators of Disturbance in Neotropical Rainforests. *Conservation Biology*, 14(6), 1666–1675.

Murray, K. a & Daszak, P. (2013). Human Ecology in Pathogenic Landscapes: Two Hypotheses on How Land Use Change Drives Viral Emergence. *Current opinion in virology*, 3(1), 79–83.

Ojeda-Flores, R., Anthony, S. J., Santiago-Alarcon, D., Rico-Chávez, O., Sarmiento-Silva, R. E., González-Santillán, E., et al. Bat-Coronavirus Co - Phylogenetic Patterns in Mexico Indicate Strong Host Specificity. *PloS one*, In Review.

Oksanen, J., Blanchet, G. F., Kindt, R., Legendre, P., Minchin, P. R., O´Hara, R. B., et al. (2013). Vegan: Community Ecology Package.

Olson, S. H., Parmley, J., Soos, C., Gilbert, M., Latorre-Margalef, N., Hall, J. S., et al. (2014). Sampling Strategies and Biodiversity of Influenza a Subtypes in Wild Birds. *PloS one*, 9(3), e90826.

Patz, J. A., Daszak, P., Tabor, G. M., Aguirre, A. A., Pearl, M., Epstein, J., et al. (2004). Unhealthy Landscapes: Policy Recommendations on Land Use Change and Infectious Disease Emergence. *Environmental Health Perspectives*, 112, 1092–1098.

Poulin, R., Krasnov, B. R. & Mouillot, D. (2011). Host Specificity in Phylogenetic and Geographic Space. *Trends in parasitology*, 27(8), 355–61.

Poulin, R. & Mouillot, D. (2003). Parasite Specialization from a Phylogenetic Perspective: A New Index of Host Specificity. *Parasitology*, 126(Pt 5), 473–80. Retrieved October 8, 2014, from http://www.ncbi.nlm.nih.gov/pubmed/12793652

Quan, P.-L., Firth, C., Conte, J. M., Williams, S. H., Zambrana-Torrelio, C. M., Anthony, S. J., et al. (2013). Bats Are a Major Natural Reservoir for

Hepaciviruses and Pegiviruses. *Proceedings of the National Academy of Sciences of the United States of America*, 110(20), 8194–9.

R Core Team. (2013). R: A Language and Environment for Statistical Computing.

Rivard, D. H., Poitevin, J., Plasse, D., Carleton, M., Currie, D. J., Ward, D. H., et al. (2007). Changing Species Richness and Composition in Canadian National Parks. , 14(4), 1099–1109.

Rubio, A. V, Avila-Flores, R. & Suzán, G. (2014). Responses of Small Mammals to Habitat Fragmentation: Epidemiological Considerations for Rodent-Borne Hantaviruses in the Americas. *EcoHealth*.

Scordato, E. S. C. & Kardish, M. R. (2014). Prevalence and Beta Diversity in Avian Malaria Communities: Host Species Is a Better Predictor than Geography. *The Journal of animal ecology*.

Shannon, C. E. (1948). A Mathematical Theory of Communication. *System Technical Journal*, (27), 379–423.

Smith, I., Broos, A., de Jong, C., Zeddeman, A., Smith, C., Smith, G., et al. (2011). Identifying Hendra Virus Diversity in Pteropid Bats. *PLoS ONE*, 6(9).

Streicker, D. G., Turmelle, A. S., Vonhof, M. J., Kuzmin, I. V, McCracken, G. F. & Rupprecht, C. E. (2010). Host Phylogeny Constrains Cross-Species Emergence and Establishment of Rabies Virus in Bats. *Science (New York, N.Y.)*, 329(5992), 676–9.

Suzán, G., Marcé, E., Giermakowski, J. T., Mills, J. N., Ceballos, G., Ostfeld, R. S., et al. (2009). Experimental Evidence for Reduced Rodent Diversity Causing Increased Hantavirus Prevalence. *PloS one*, 4(5), e5461.

Svensson-Coelho, M. & Ricklefs, R. E. (2011). Host Phylogeography and Beta Diversity in Avian Haemosporidian (Plasmodiidae) Assemblages of the Lesser Antilles. *The Journal of animal ecology*, 80(5), 938–46.

Tews, J., Brose, U., Grimm, V., Tielbörger, K., Wichmann, M. C., Schwager, M., et al. (2004). Animal Species Diversity Driven by Habitat Heterogeneity/diversity: The Importance of Keystone Structures. *Journal of Biogeography*, 31(1), 79–92.

Tong, S., Chern, S.-W. W., Li, Y., Pallansch, M. A. & Anderson, L. J. (2008). Sensitive and Broadly Reactive Reverse Transcription-PCR Assays to Detect Novel Paramyxoviruses. *Journal of clinical microbiology*, 46(8), 2652–8.

Towner, J. S., Amman, B. R., Sealy, T. K., Reeder Carroll, S. A., Comer, J. A., Kemp, A., et al. (2009). Isolation of Genetically Diverse Marburg Viruses from Egyptian Fruit Bats. *PLoS Pathogens*, 5.

Ulrich, D. H., Almeida-Neto, M. & Gotelli, N. J. (2009). A Consumer's Guide to Nestedness Analysis. *Oikos*, 118, 3–17.

Webb, C. O., Ackerly, D. D., McPeek, M. a. & Donoghue, M. J. (2002). Phylogenies and Community Ecology. *Annual Review of Ecology and Systematics*, 33(1), 475–505.

Woolhouse, M. E. J. & Gowtage-Sequeria, S. (2005). Host Range and Emerging and Reemerging Pathogens. *Emerging infectious diseases*, 11(12), 1842–7.

Discusión general

Antes de este estudio, se conocía muy poco acerca de la diversidad de CoVs en el Neotrópico, a pesar de la gran diversidad de especies de murciélagos que se encuentran en esta región (Wilson & Reeder, 2005). Con este estudio se demostró la existencia de varios virus adicionales correspondientes a los géneros *Alfacoronavirus* y *Betacoronavirus* en México. Las secuencias detectadas fueron añadidas a *Genbank* con los números KC117184-KC117213. Los análisis filogenéticos del fragmento de la polimerasa viral muestran que los CoVs se agrupan con base en la relación filogenética de las especies hospederas acorde a las hipótesis planteadas al inicio del proyecto. Se analizó por primera vez la historia cofilogenética entre murciélagos del Nuevo Mundo y α-CoVs y β-CoVs, en respuesta a la recientemente señalada falta de información de sitios *hotspot* con alta biodiversidad que incluyen la región Neotropical (Drexler *et al.*, 2014). Se detectó una fuerte señal de coespeciación entre la historia evolutiva de los murciélagos del Nuevo Mundo y sus respectivos CoVs usando dos metodologías complementarias. Al realizar análisis separando α-CoVs y β -CoVs también se detectaron patrones cofilogenéticos estadísticamente significativos. Algunas asociaciones particulares entre CoVs y ciertas especies de murciélagos determinan mayormente el patrón global de cofilogenia, en particular aquellas que involucran a los géneros de murciélagos *Artibeus* y *Carollia*. Los resultados sugieren la existencia de un alto grado de especificidad de hospedero a nivel de género (con cambios relativamente frecuentes entre murciélagos del mismo género), con cierto grado de diversificación entre hospederos (diversificación intra-hospedero).

A través de la combinación de la información derivada de los árboles filogenéticos y los datos sobre el origen geográfico de los CoVs, se determinó la distribución filogeográfica de algunos CoVs mexicanos. Esta información demuestra que la

diversificación de los CoVs en murciélagos neotropicales es compleja, sugiriendo que tanto la especiación intra-hospedero, como los cambios de hospedero, en combinación con el aislamiento geográfico, son mecanismos importantes en la especiación de los CoVs. El hecho de que estos virus cambien esporádicamente entre murciélagos de diferente género sugiere que la historia evolutiva de estos sistemas parásito-hospedero es dominada por rasgos del hospedero (por ejemplo, características inmunológicas) que son conservados a niveles taxonómicos inferiores (género). En ausencia de barreras ecológicas importantes que eviten los cambios de hospedero, los linajes de virus relacionados infectan grupos relacionados de hospederos.

La prevalencia de CoVs y las dinámicas de infección pueden ser afectadas por factores filogeográficos y ambientales asociados a los procesos de coespeciación parásito-hospedero (Davies & Pedersen, 2008). Los resultados de este estudio sugieren que el clado monofilético correspondiente a la Ciudad de México es filogenética y geográficamente distinto. Las razones específicas de este patrón se desconocen, pero podrían reflejar diferencias en la diversidad (riqueza y abundancia relativa) y la composición de las especies de murciélagos entre la región Neártica (Ciudad de México) y la región Neotropical (Campeche y Chiapas). Las regiones neotropicales generalmente albergan una mayor riqueza de especies, mientras que la abundancia de cada especie suele ser baja. En contraste, las regiones neárticas suelen caracterizarse por una riqueza general menor, pero contienen mayor número de individuos por especie (Ortega & Arita, 1998). La zona de transición entre estas dos regiones biogeográficas representa una zona evolutivamente "activa", donde varios eventos de especiación han tenido lugar en el pasado. Esto ha propiciando que México sea reconocido como una región de alta diversidad y endemismo, en la que coexisten especies residentes, migratorias y endémicas (Escalante *et al.*, 2004; Marshall & Liebherr, 2000; Ortega & Arita, 1998). Es necesario aumentar los esfuerzos de muestreo en estas regiones con el fin de investigar cómo la diversidad de especies y la composición

de las comunidades de murciélagos varía entre las diferentes áreas biogeográficas, para descubrir y caracterizar la diversidad y la prevalencia de CoVs asociados a murciélagos, y luego explorar las influencias ecológicas y filogeográficas en los patrones de coespeciación entre CoVs y murciélagos de esta importante zona de transición.

Se ha propuesto que el genoma considerablemente largo y las altas tasas de mutación y recombinación, predisponen a los CoVs a saltos taxonómicos (Woo *et al.*, 2009a), resaltando la existencia tanto de virus “promiscuos” como de virus altamente específicos dentro de la filogenia de los CoVs (Drexler *et al.*, 2014). Clasificados en el género β -CoVs, los CoVs relacionados al SARS, y más recientemente, los relacionados al MERS (detectados en todo el mundo, incluyendo el señalado en el presente estudio), han recibido mucha atención debido a sus implicaciones en salud pública. Notablemente, la mayoría de los CoVs detectados en la región biogeográfica Neotropical (Anthony *et al.*, 2013a; Corman *et al.*, 2013) se encuentran restringidos a un solo género de hospedero, incluyendo detecciones de CoVs estrechamente relacionados infectando poblaciones de murciélagos separadas por varios cientos de kilómetros. La congruencia observada a través de los análisis cofilogenéticos utilizando este modelo parásito-hospedero, sugiere que los CoVs mexicanos y sus anfitriones quirópteros comparten una historia de diversificación dominada por eventos de coespeciación con cierto grado de diversificación asociada a saltos taxonómicos entre especies estrechamente relacionadas, pero poco frecuente entre murciélagos de diferente género. Por lo tanto, con base en esta información, se sugiere que los CoVs de murciélagos mexicanos tienen una baja probabilidad de convertirse en zoonóticos, y que las diferencias en el sistema inmune de las especies hospederas juegan un papel más importante en la determinación de la especificidad en comparación con los factores ecológicos, puesto que la mayoría de la especies hospederas incluidas en este estudio comparten el mismo nicho que proporciona amplias oportunidades para la ocurrencia de saltos taxonómicos.

En trabajos posteriores, distintos grupos de investigación realizaron estudios colectando y/o analizando muestras y/o secuencias de murciélagos en busca de CoVs. Los análisis a nivel filogenético han permitido acumular una creciente cantidad de información que soporta la existencia mundial de CoVs relacionados al MERS en murciélagos. Estos análisis han corroborado la aproximación del presente estudio, ya que consistentemente se han identificado secuencias virales que presumiblemente corresponden a antecesores del MERS-CoV en quirópteros de las familias *Molossidae* y *Vespertilionidae*. Estos CoVs incluyen el virus BtVs-BetaCoV/SC2013, detectado en murciélagos *Vespertilio superans* de China (Yang *et al.*, 2014); murciélagos *Pipistrellus* de Europa; un virus denominado PML/2011, detectado en *Neoromicia zuluensis* de Sudáfrica (Annan *et al.*, 2013; Ithete *et al.*, 2013) y secuencias detectadas en muestras de guano de murciélagos de Tailandia (Wacharapluesadee *et al.*, 2013). Así como CoVs de murciélagos *Hypsugo savii* y *Eptesicus isabellinus*, muestreados con anterioridad en España (Falcon *et al.*, 2011) y los β -CoVs HKU4 y HKU5, procedentes de muestras colectadas previamente en China (Woo *et al.*, 2007). Además de un fragmento de 203 nucleótidos 100% idéntico a la secuencia MERS-CoV correspondiente a la RdRp denominada EMC/2012, la cual fue descubierta en un *Taphozous perforatus* de Arabia Saudita*,* murciélago que pertenece a la familia Emballonuridae (Memish *et al.*, 2013). Considerando la estrecha relación filogenética entre las especies de murciélagos estudiados en estos trabajos y el alto grado de identidad de los CoVs emparentados con el MERS-CoV, en conjunto, estos datos refuerzan la idea de asociación entre la especiación de los murciélagos hospederos y los correspondientes CoVs.

De entre las distintas aproximaciones aplicables al estudio de la emergencia de enfermedades zoonóticas, el enfoque basado en asociaciones evolutivas entre hospederos y parásitos podría ser de gran utilidad en la identificación de reservorios virales relacionados a saltos inter-especie, bajo el supuesto de que un

virus emergente comparte suficiente similitud con los virus circulantes en comunidades de reservorios, resaltando la importancia del estudio sistemático de comunidades de hospederos y reservorios desde perspectivas ecológicas y evolutivas. La comprensión de la complejidad de las interacciones que subyacen la emergencia de enfermedades zoonóticas se ha visto superada por los enfoques epidemiológicos tradicionales, que estudian constantemente los aspectos del agente etiológico, por lo general suponiendo que no hay interacción con otros patógenos. La exclusión del estudio de las comunidades de parásitos y ciertas características de la asociación parásito-hospedero que ocurren a niveles ecológico y evolutivo es el resultado de la simplificación excesiva que caracteriza los enfoques basados en un solo hospedero y/o un solo parásito. La investigación acerca de la dinámica de las EIE que conduzca a la comprensión de las interacciones entre patógenos y hospederos tanto de fauna silvestre como doméstica, sólo se puede desarrollar mediante la integración de estudios sobre las causas asociadas a la emergencia de enfermedades, combinando esfuerzos interdisciplinarios a través de las ciencias biológicas, físicas y sociales, a fin de mejorar las estrategias de control basadas en la integración de este conocimiento.

La atención científica actual sobre el inexorable vínculo que existe entre la salud humana, animal y las funciones ecosistémicas, resultado de la creciente tasa de emergencia de enfermedades zoonóticas se relaciona estrechamente con la expansión agrícola y la intensificación de la producción pecuaria. Esta conexión destaca la responsabilidad de la ciencia veterinaria en evaluar, predecir, prevenir y responder a los riesgos de zoonosis en la interfaz fauna silvestre-doméstica-población humana desde un enfoque multidisciplinario. De igual forma, es necesario desarrollar nuevos esquemas de prevención y respuesta ante el surgimiento de EIE en humanos, animales de producción y fauna silvestre que combinen el conocimiento científico con los planes gubernamentales en los marcos actuales de estudio y acción mundiales. La escasa incidencia de la producción científica nacional relacionada a la ecología de enfermedades sobre

las decisiones políticas limita la toma de decisiones, al tiempo que retrasa las acciones necesarias para desarrollar sistemas agropecuarios sostenibles que satisfagan las necesidades alimentarias de la creciente población nacional, y al mismo tiempo reduzcan el riesgo de emergencia de enfermedades protegiendo la salud pública, la producción animal y la soberanía alimentaria, preservando la biodiversidad y las funciones de los ecosistemas de México.

Conocer los virus existentes a nivel mundial es de primordial importancia para el análisis y comprensión del *pool* con potencial zoonótico del que se produce la emergencia de enfermedades. En consecuencia, el estudio y descripción de esta abundancia y diversidad virales de una manera sistemática es esencial para la comprensión de los procesos y patrones actuales, así como los mecanismos que conducen a dicha emergencia. Hasta ahora, la mayoría de los estudios enfocados al descubrimiento de virus han considerado muy pobremente el vínculo entre la abundancia/diversidad viral y la ecología de los hospederos, a pesar de la evidente relación con el riesgo de emergencia viral. Aquí hemos tratado de abordar esta filosofía multidisciplinaria y hemos combinado los enfoques comunes a la ecología y la epidemiología molecular en nuestra descripción de estos nuevos coronavirus de México.

Referencias

Annan, A., Baldwin, H. J., Corman, V. M., Klose, S. M., Owusu, M., Nkrumah, E. E., Badu, E. K., Anti, P., Agbenyega, O. & other authors (2013). Human betacoronavirus 2c EMC/2012-related viruses in bats, Ghana and Europe. *Emerging infectious diseases* **19**, 456-459.

Anthony, S. J., Ojeda-Flores, R., Rico-Chavez, O., Navarrete-Macias, I., Zambrana-Torrelio, C. M., Rostal, M. K., Epstein, J. H., Tipps, T., Liang, E. & other authors (2013a). Coronaviruses in bats from Mexico. *The Journal of general virology* **94**, 1028-1038.

Anthony, S. J., Epstein, J. H., Murray, K. A., Navarrete-Macias, I., Zambrana-Torrelio, C. M., Solovyov, A., Ojeda-Flores, R., Arrigo, N. C., Islam, A. & other authors (2013b). A strategy to estimate unknown viral diversity in mammals. *mBio* **4**, e00598-00513.

Arias, C. F. (2013). Virus diversity and evolution. *Current opinion in microbiology* **16**, 465-467.

Arnaud, F., Caporale, M., Varela, M., Biek, R., Chessa, B., Alberti, A., Golder, M., Mura, M., Zhang, Y. P. & other authors (2007). A paradigm for virus-host coevolution: sequential counter-adaptations between endogenous and exogenous retroviruses. *PLoS pathogens* **3**, e170.

August, T. A., Mathews, F. & Nunn, M. A. (2012). Alphacoronavirus detected in bats in the United Kingdom. *Vector-Borne Zoonotic Diseases* **12**, 530-533.

Balboni, A., Palladini, A., Bogliani, G. & Battilani, M. (2011). Detection of a virus related to betacoronaviruses in Italian greater horseshoe bats. *Epidemiology and infection* **139**, 216-219.

Balboni, A., Gallina, L., Palladini, A., Prosperi, S. & Battilani, M. (2012). A real-time PCR assay for bat SARS-like coronavirus detection and its application to Italian greater horseshoe bat faecal sample surveys. *ScientificWorldJournal* **2012**, 989514.

Bausch, D. G. & Schwarz, L. (2014). Outbreak of ebola virus disease in Guinea: where ecology meets economy. *PLoS neglected tropical diseases* **8**, e3056.

Bennett, S. N., Gu, S. H., Kang, H. J., Arai, S. & Yanagihara, R. (2014). Reconstructing the evolutionary origins and phylogeography of hantaviruses. *Trends in microbiology* **22**, 473-482.

Beutels, P., Jia, N., Zhou, Q. Y., Smith, R., Cao, W. C. & de Vlas, S. J. (2009). The economic impact of SARS in Beijing, China. *Tropical medicine & international health : TM & IH* **14 Suppl 1**, 85-91.

Bininda-Emonds, O. R., Cardillo, M., Jones, K. E., MacPhee, R. D., Beck, R. M., Grenyer, R., Price, S. A., Vos, R. A., Gittleman, J. L. & other authors (2007). The delayed rise of present-day mammals. *Nature* **446**, 507-512.

Calisher, C. H., Childs, J. E., Field, H. E., Holmes, K. V. & Schountz, T. (2006). Bats: important reservoir hosts of emerging viruses. *Clin Microbiol Rev* **19**, 531-545.

Carrington, C. V., Foster, J. E., Zhu, H. C., Zhang, J. X., Smith, G. J., Thompson, N., Auguste, A. J., Ramkissoon, V., Adesiyun, A. A. & other authors (2008). Detection and phylogenetic analysis of group 1 coronaviruses in South American bats. *Emerging Infectious Disease* **14**, 1890-1893.

Cavanagh, D. (2005). Coronaviruses in poultry and other birds. *Avian Pathology* **34**, 439-448.

Chu, D. K., Peiris, J. S., Chen, H., Guan, Y. & Poon, L. L. (2008). Genomic characterizations of bat coronaviruses (1A, 1B and HKU8) and evidence for co-infections in Miniopterus bats. *The Journal of general virology* **89**, 1282-1287.

Chu, D. K., Poon, L. L., Chan, K. H., Chen, H., Guan, Y., Yuen, K. Y. & Peiris, J. S. (2006). Coronaviruses in bent-winged bats (Miniopterus spp.). *The Journal of general virology* **87**, 2461-2466.

Chu, D. K. W., Peiris, J. S. M. & Poon, L. L. M. (2009). Novel coronaviruses and astroviruses in bats. *Virologica Sinica* **24**, 100-104.

Chu, D. K. W., Leung, C. Y. H., Gilbert, M., Joyner, P. H., Ng, E. M., Tse, T. M., Guan, Y., Peiris, J. S. M. & Poon, L. L. M. (2011). Avian coronavirus in wild aquatic birds. *Journal of Virology* **85**, 12815-12820.

Chua, K. B., Bellini, W. J., Rota, P. A., Harcourt, B. H., Tamin, A., Lam, S. K., Ksiazek, T. G., Rollin, P. E., Zaki, S. R. & other authors (2000). Nipah virus: a recently emergent deadly paramyxovirus. *Science* **288**, 1432-1435.

Corman, V. M., Rasche, A., Diallo, T. D., Cottontail, V. M., Stocker, A., Souza, B. F., Correa, J. I., Carneiro, A. J., Franke, C. R. & other authors (2013). Highly diversified coronaviruses in neotropical bats. *The Journal of general virology*.

Daszak, P., Cunningham, A. A. & Hyatt, A. D. (2001). Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Trop* **78**, 103-116.

Daszak, P., Zambrana-Torrelio, C., Bogich, T. L., Fernandez, M., Epstein, J. H., Murray, K. A. & Hamilton, H. (2013). Interdisciplinary approaches to understanding disease emergence: the past, present, and future drivers of Nipah virus emergence. *Proceedings of the National Academy of Sciences of the United States of America* **110 Suppl 1**, 3681-3688.

Davies, T. J. & Pedersen, A. B. (2008). Phylogeny and geography predict pathogen community similarity in wild primates and humans. *Proceedings Biological sciences / The Royal Society* **275**, 1695-1701.

De Benedictis, P., Marciano, S., Scaravelli, D., Priori, P., Zecchin, B., Capua, I., Monne, I. & Cattoli, G. (2014). Alpha and lineage C betaCoV infections in Italian bats. *Virus genes* **48**, 366-371.

de Groot, R. J., Baker, S. C., Baric, R. S., Brown, C. S., Drosten, C., Enjuanes, L., Fouchier, R. A., Galiano, M., Gorbalenya, A. E. & other authors (2013). Middle East respiratory syndrome coronavirus (MERS-CoV): announcement of the Coronavirus Study Group. *Journal of virology* **87**, 7790-7792.

Dominguez, S. R., O'Shea, T. J., Oko, L. M. & Holmes, K. V. (2007). Detection of group 1 coronaviruses in bats in North America. *Emerging Infectious Diseases* **13**, 1295-1300.

Dong, B. Q., Liu, W., Fan, X. H., Vijaykrishna, D., Tang, X. C., Gao, F., Li, L. F., Li, G. J., Zhang, J. X. & other authors (2007). Detection of a novel and highly divergent coronavirus from Asian leopard cats and Chinese ferret badgers in southern China. *Journal of Virology* **81**, 6920-6926.

Drexler, J. F., Corman, V. M. & Drosten, C. (2014). Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS. *Antiviral research* **101**, 45-56.

Drexler, J. F., Corman, V. M., Wegner, T., Tateno, A. F., Zerbinati, R. M., Gloza-Rausch, F., Seebens, A., Muller, M. A. & Drosten, C. (2011). Amplification of emerging viruses in a bat colony. *Emerging Infectious Diseases* **17**, 449-456.

Drexler, J. F., Gloza-Rausch, F., Glende, J., Corman, V. M., Muth, D., Goettsche, M., Seebens, A., Niedrig, M., Pfefferle, S. & other authors (2010). Genomic characterization of Severe Acute Respiratory Syndrome-Related coronavirus in European bats and classification of coronaviruses based on partial RNA-dependent RNA polymerase gene sequences. *Journal of Virology* **84**, 11336-11349.

Drexler, J. F., Corman, V. M., Muller, M. A., Maganga, G. D., Vallo, P., Binger, T., Gloza-Rausch, F., Cottontail, V. M., Rasche, A. & other authors (2012). Bats host major mammalian paramyxoviruses. *Nature communications* **3**, 796.

Drosten, C., Gunther, S., Preiser, W., van der Werf, S., Brodt, H. R., Becker, S., Rabenau, H., Panning, M., Kolesnikova, L. & other authors (2003). Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *The New England journal of medicine* **348**, 1967-1976.

Escalante, T., Rodríguez, G. & J., M. J. (2004). The diversification of Nearctic mammals in the Mexican transition zone. *Biological Journal of the Linnean Society* **83**, 327-339.

Ezenwa, V. O., Godsey, M. S., King, R. J. & Guptill, S. C. (2006). Avian diversity and West Nile virus: testing associations between biodiversity and infectious disease risk. *Proceedings Biological sciences / The Royal Society* **273**, 109-117.

Falcon, A., Vazquez-Moron, S., Casas, I., Aznar, C., Ruiz, G., Pozo, F., Perez-Brena, P., Juste, J., Ibanez, C. & other authors (2011). Detection of alpha and betacoronaviruses in multiple Iberian bat species. *Archives of virology* **156**, 1883-1890.

Felippe, P. A. N., da Silva, L. H. A., Santos, M., Spilki, F. R. & Arns, C. W. (2010). Genetic diversity of Avian Infectious Bronchitis Virus isolated from domestic chicken flocks and coronaviruses from feral pigeons in Brazil between 2003 and 2009. *Avian Diseases* **54**, 1191-1196.

Fonkwo, P. N. (2008). Pricing infectious disease. The economic and health implications of infectious diseases. *EMBO Rep* **9 Suppl 1**, S13-17.

Freuling, C. M., Beer, M., Conraths, F. J., Finke, S., Hoffmann, B., Keller, B., Kliemt, J., Mettenleiter, T. C., Muhlbach, E. & other authors (2011). Novel lyssavirus in Natterer's bat, Germany. *Emerging infectious diseases* **17**, 1519-1522.

Ge, X., Li, Y., Yang, X., Zhang, H., Zhou, P., Zhang, Y. & Shi, Z. (2012). Metagenomic analysis of viruses from bat fecal samples reveals many novel viruses in insectivorous bats in China. *Journal of virology* **86**, 4620-4630.

Ge, X. Y., Li, J. L., Yang, X. L., Chmura, A. A., Zhu, G., Epstein, J. H., Mazet, J. K., Hu, B., Zhang, W. & other authors (2013). Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature* **503**, 535-538.

Geldenhuys, M., Weyer, J., Nel, L. H. & Markotter, W. (2013). Coronaviruses in South african bats. *Vector borne and zoonotic diseases* **13**, 516-519.

Gloza-Rausch, F., Ipsen, A., Seebens, A., Gottsche, M., Panning, M., Drexler, J. F., Petersen, N., Annan, A., Grywna, K. & other authors (2008). Detection and prevalence patterns of group I coronaviruses in bats, northern Germany. *Emerging Infectious Diseases* **14**, 626-631.

Goes, L. G., Ruvalcaba, S. G., Campos, A. A., Queiroz, L. H., de Carvalho, C., Jerez, J. A., Durigon, E. L., Davalos, L. I. & Dominguez, S. R. (2013). Novel bat coronaviruses, Brazil and Mexico. *Emerging infectious diseases* **19**, 1711-1713.

Gouilh, M. A., Puechmaille, S. J., Gonzalez, J.-P., Teeling, E., Kittayapong, P. & Manuguerra, J.-C. (2011). SARS-Coronavirus ancestor's foot-prints in South-East Asian bat colonies and the refuge theory. *Infection, Genetics and Evolution* **11**, 1690-1702.

Guan, Y., Zheng, B. J., He, Y. Q., Liu, X. L., Zhuang, Z. X., Cheung, C. L., Luo, S. W., Li, P. H., Zhang, L. J. & other authors (2003). Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science* **302**, 276-278.

Guernier, V., Hochberg, M. E. & Guegan, J. F. (2004). Ecology drives the worldwide distribution of human diseases. *PLoS biology* **2**, e141.

Haagmans, B. L., Andeweg, A. C. & Osterhaus, A. D. (2009). The application of genomics to emerging zoonotic viral diseases. *PLoS pathogens* **5**, e1000557.

He, B., Zhang, Y., Xu, L., Yang, W., Yang, F., Feng, Y., Xia, L., Zhou, J., Zhen, W. & other authors (2014). Identification of diverse alphacoronaviruses and genomic characterization of a novel severe acute respiratory syndrome-like coronavirus from bats in China. *Journal of virology* **88**, 7070-7082.

Hilgenfeld, R. & Peiris, M. (2013). From SARS to MERS: 10 years of research on highly pathogenic human coronaviruses. *Antiviral research* **100**, 286-295.

Huynh, J., Li, S., Yount, B., Smith, A., Sturges, L., Olsen, J. C., Nagel, J., Johnson, J. B., Agnihothram, S. & other authors (2012). Evidence supporting a zoonotic origin of human coronavirus strain NL63. *Journal of virology* **86**, 12816-12825.

Ithete, N. L., Stoffberg, S., Corman, V. M., Cottontail, V. M., Richards, L. R., Schoeman, M. C., Drosten, C., Drexler, J. F. & Preiser, W. (2013). Close relative of human Middle East respiratory syndrome coronavirus in bat, South Africa. *Emerging infectious diseases* **19**, 1697-1699.

Jackwood, M. W., Hall, D. & Handel, A. (2012). Molecular evolution and emergence of avian gammacoronaviruses. *Infection, Genetics and Evolution* **12**, 1305-1311.

Jia, G. L., Zhang, Y., Wu, T. H., Zhang, S. Y. & Wang, Y. N. (2003). Fruit bats as a natural reservoir of zoonotic viruses. *Chinese Science Bulletin* **48**, 1179-1182.

Johnson, P. T., Preston, D. L., Hoverman, J. T. & Richgels, K. L. (2013). Biodiversity decreases disease through predictable changes in host community competence. *Nature* **494**, 230-233.

Jones, B. A., Grace, D., Kock, R., Alonso, S., Rushton, J., Said, M. Y., McKeever, D., Mutua, F., Young, J. & other authors (2013). Zoonosis emergence linked to agricultural intensification and environmental change. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 8399-8404.

Jones, K. E., Bininda-Emonds, O. R. & Gittleman, J. L. (2005). Bats, clocks, and rocks: diversification patterns in Chiroptera. *Evolution; international journal of organic evolution* **59**, 2243-2255.

Jones, K. E., Patel, N. G., Levy, M. A., Storeygard, A., Balk, D., Gittleman, J. L. & Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature* **451**, 990-993.

Karesh, W. B., Dobson, A., Lloyd-Smith, J. O., Lubroth, J., Dixon, M. A., Bennett, M., Aldrich, S., Harrington, T., Formenty, P. & other authors (2012). Ecology of zoonoses: natural and unnatural histories. *Lancet* **380**, 1936-1945.

Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., Hudson, P., Jolles, A., Jones, K. E. & other authors (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature* **468**, 647-652.

Ksiazek, T., Erdman, D., Goldsmith, C., Zaki, S., Peret, T. & Emery, S. (2003). A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* **348**, 1953-1966.

Kuiken, T., Leighton, F. A., Fouchier, R. A., LeDuc, J. W., Peiris, J. S., Schudel, A., Stohr, K. & Osterhaus, A. D. (2005). Public health. Pathogen surveillance in animals. *Science* **309**, 1680-1681.

Lau, S. K., Woo, P. C., Li, K. S., Huang, Y., Tsoi, H. W., Wong, B. H., Wong, S. S., Leung, S. Y., Chan, K. H. & other authors (2005). Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 14040-14045.

Lau, S. K., Woo, P. C., Li, K. S., Huang, Y., Wang, M., Lam, C. S., Xu, H., Guo, R., Chan, K. H. & other authors (2007). Complete genome sequence of bat coronavirus HKU2 from Chinese horseshoe bats revealed a much smaller spike gene with a different evolutionary lineage from the rest of the genome. *Virology* **367**, 428-439.

Lau, S. K., Poon, R. W., Wong, B. H., Wang, M., Huang, Y., Xu, H., Guo, R., Li, K. S., Gao, K. & other authors (2010a). Coexistence of different genotypes in the same bat and serological characterization of Rousettus bat coronavirus HKU9 belonging to a novel Betacoronavirus subgroup. *Journal of virology* **84**, 11385-11394.

Lau, S. K., Li, K. S., Tsang, A. K., Shek, C. T., Wang, M., Choi, G. K., Guo, R., Wong, B. H., Poon, R. W. & other authors (2012a). Recent transmission of a novel alphacoronavirus, bat coronavirus HKU10, from Leschenault's rousettes to pomona leaf-nosed bats: first evidence of interspecies transmission of coronavirus between bats of different suborders. *Journal of virology* **86**, 11906-11918.

Lau, S. K. P., Li, K. S. M., Huang, Y., Shek, C. T., Tse, H., Wang, M., Choi, G. K. Y., Xu, H. F., Lam, C. S. F. & other authors (2010b). Ecoepidemiology and complete genome comparison of different strains of Severe Acute Respiratory Syndrome-related *Rhinolophus* bat coronavirus in China reveal bats as a reservoir for acute, self-limiting infection that allows recombination events. *Journal Of Virology* **84**, 2808-2819.

Lau, S. K. P., Woo, P. C. Y., Yip, C. C. Y., Fan, R. Y. Y., Huang, Y., Wang, M., Guo, R. T., Lam, C. S. F., Tsang, A. K. L. & other authors (2012b). Isolation and characterization of a novel *Betacoronavirus* subgroup A coronavirus, Rabbit Coronavirus HKU14, from domestic rabbits. *Journal of Virology* **86**, 5481-5496.

Lei, B. R. & Olival, K. J. (2014). Contrasting patterns in mammal-bacteria coevolution: bartonella and leptospira in bats and rodents. *PLoS neglected tropical diseases* **8**, e2738.

Lelli, D., Papetti, A., Sabelli, C., Rosti, E., Moreno, A. & Boniotti, M. B. (2013). Detection of coronaviruses in bats of various species in Italy. *Viruses* **5**, 2679-2689.

Leroy, E. M., Kumulungui, B., Pourrut, X., Rouquet, P., Hassanin, A., Yaba, P., Delicat, A., Paweska, J. T., Gonzalez, J. P. & other authors (2005). Fruit bats as reservoirs of Ebola virus. *Nature* **438**, 575-576.

Levinson, J., Bogich, T. L., Olival, K. J., Epstein, J. H., Johnson, C. K., Karesh, W. & Daszak, P. (2013). Targeting surveillance for zoonotic virus discovery. *Emerging infectious diseases* **19**, 743-747.

Lewis-Rogers, N. & Crandall, K. A. (2010). Evolution of Picornaviridae: an examination of phylogenetic relationships and cophylogeny. *Molecular phylogenetics and evolution* **54**, 995-1005.

Li, L., Victoria, J. G., Wang, C., Jones, M., Fellers, G. M., Kunz, T. H. & Delwart, E. (2010). Bat guano virome: predominance of dietary viruses from

insects and plants plus novel mammalian viruses. *Journal of virology* **84**, 6955-6965.

Li, W., Shi, Z., Yu, M., Ren, W., Smith, C., Epstein, J. H., Wang, H., Crameri, G., Hu, Z. & other authors (2005). Bats are natural reservoirs of SARS-like coronaviruses. *Science* **310**, 676-679.

Lima, F. E., Campos, F. S., Kunert Filho, H. C., Batista, H. B., Carnielli Junior, P., Cibulski, S. P., Spilki, F. R., Roehe, P. M. & Franco, A. C. (2013). Detection of Alphacoronavirus in velvety free-tailed bats (Molossus molossus) and Brazilian free-tailed bats (Tadarida brasiliensis) from urban area of Southern Brazil. *Virus genes*.

Lipkin, W. I. & Firth, C. (2013). Viral surveillance and discovery. *Current opinion in virology* **3**, 199-204.

Lobo, F. P., Mota, B. E., Pena, S. D., Azevedo, V., Macedo, A. M., Tauch, A., Machado, C. R. & Franco, G. R. (2009). Virus-host coevolution: common patterns of nucleotide motif usage in Flaviviridae and their hosts. *PloS one* **4**, e6282.

Luis, A. D., Hayman, D. T., O'Shea, T. J., Cryan, P. M., Gilbert, A. T., Pulliam, J. R., Mills, J. N., Timonin, M. E., Willis, C. K. & other authors (2013). A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special? *Proceedings Biological sciences / The Royal Society* **280**, 20122753.

Mackey, T. K. & Liang, B. A. (2012). Lessons from SARS and H1N1/A: employing a WHO-WTO forum to promote optimal economic-public health pandemic response. *Journal of public health policy* **33**, 119-130.

Maganga, G. D., Bourgarel, M., Vallo, P., Dallo, T. D., Ngoagouni, C., Drexler, J. F., Drosten, C., Nakoune, E. R., Leroy, E. M. & other authors (2014). Bat distribution size or shape as determinant of viral richness in african bats. *PloS one* **9**, e100172.

Mann, B. R., McMullen, A. R., Guzman, H., Tesh, R. B. & Barrett, A. D. (2013). Dynamic transmission of West Nile virus across the United States-Mexican border. *Virology* **436**, 75-80.

Marshall, C. J. & Liebherr, J. K. (2000). Cladistic Biogeography of the Mexican Transition Zone. *Journal of Biogeography* **27**, 203-216.

Martínez-Meyer, E., Sosa-Escalante, J. & Álvarez, F. (2014). The study of the biodiversity in Mexico: a route with a course? *Revista Mexicana de Biodiversidad, Supl 85: S1-S9, 2014* **Supl. 85: S1-S9, 2014**, S1-S9.

Mayer, F., Dietz, C. & Kiefer, A. (2007). Molecular species identification boosts bat diversity. *Frontiers in zoology* **4**, 4.

Memish, Z. A., Mishra, N., Olival, K. J., Fagbo, S. F., Kapoor, V., Epstein, J. H., Alhakeem, R., Durosinloun, A., Al Asmari, M. & other authors (2013). Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. *Emerging infectious diseases* **19**, 1819-1823.

Misra, V., Dumonceaux, T., Dubois, J., Willis, C., Nadin-Davis, S., Severini, A., Wandeler, A., Lindsay, R. & Artsob, H. (2009). Detection of polyoma and corona viruses in bats of Canada. *Journal of General Virology* **90**, 2015-2022.

Mittermeier, R. A., Mast, R. B., del Prado, C. P. & Mittermeier, C. G. (1997). *Megadiversity: Earth's Biologically Wealthiest Nations*. Mexico: Conservation international, CEMEX.

Morse, S. S., Mazet, J. A., Woolhouse, M., Parrish, C. R., Carroll, D., Karesh, W. B., Zambrana-Torrelio, C., Lipkin, W. I. & Daszak, P. (2012). Prediction and prevention of the next pandemic zoonosis. *Lancet* **380**, 1956-1965.

Murray, K. A. & Daszak, P. (2013). Human ecology in pathogenic landscapes: two hypotheses on how land use change drives viral emergence. *Current opinion in virology* **3**, 79-83.

Olival, K., Epstein, J., Wang, L. & Field, H. E. (2012). Are bats exceptional viral reservoirs. In *New directions in conservation medicine: Applied cases of ecological health*, pp. 195-212.

Ortega, J. & Arita, H. T. (1998). Neotropical-Nearctic limits in Middle America as determined by distributions of bats. *Journal of Mammalogy* **79**, 772-783.

Osborne, C., Cryan, P. M., O'Shea, T. J., Oko, L. M., Ndaluka, C., Calisher, C. H., Berglund, A. D., Klavetter, M. L., Bowen, R. A. & other authors (2011). Alphacoronaviruses in New World bats: prevalence, persistence, phylogeny, and potential for interaction with humans. *PLoS ONE* **6**, e19156.

Ostfeld, R. S. (2009). Biodiversity loss and the rise of zoonotic pathogens. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* **15 Suppl 1**, 40-43.

Pfefferle, S., Oppong, S., Drexler, J. F., Gloza-Rausch, F., Ipsen, A., Seebens, A., Muller, M. A., Annan, A., Vallo, P. & other authors (2009). Distant relatives of Severe Acute Respiratory Syndrome coronavirus and close relatives of Human Coronavirus 229E in bats, Ghana. *Emerging Infectious Diseases* **15**, 1377-1384.

Pinkert, S., Klingel, K., Lindig, V., Dorner, A., Zeichhardt, H., Spiller, O. B. & Fechner, H. (2011). Virus-host coevolution in a persistently coxsackievirus B3-infected cardiomyocyte cell line. *Journal of virology* **85**, 13409-13419.

Plowright, R. K., Eby, P., Hudson, P. J., Smith, I. L., Westcott, D., Bryden, W. L., Middleton, D., Reid, P. A., McFarlane, R. A. & other authors (2015). Ecological dynamics of emerging bat virus spillover. *Proceedings Biological sciences / The Royal Society* **282**.

Poon, L. L., Chu, D. K., Chan, K. H., Wong, O. K., Ellis, T. M., Leung, Y. H., Lau, S. K., Woo, P. C., Suen, K. Y. & other authors (2005). Identification of a novel coronavirus in bats. *Journal of virology* **79**, 2001-2009.

Quan, P. L., Firth, C., Street, C., Henriquez, J. A., Petrosov, A., Tashmukhamedova, A., Hutchison, S. K., Egholm, M., Osinubi, M. O. V. & other authors (2010). Identification of a Severe Acute Respiratory Syndrome Coronavirus-Like Virus in a Leaf-Nosed Bat in Nigeria. *mBio* **1**, e00208-e00210.

Quan, P. L., Firth, C., Conte, J. M., Williams, S. H., Zambrana-Torrelio, C. M., Anthony, S. J., Ellison, J. A., Gilbert, A. T., Kuzmin, I. V. & other authors (2013). Bats are a major natural reservoir for hepaciviruses and pegiviruses. *Proceedings of the National Academy of Sciences of the United States of America* **110**, 8194-8199.

Rahman, S. A., Hassan, S. S., Olival, K. J., Mohamed, M., Chang, L. Y., Hassan, L., Saad, N. M., Shohaimi, S. A., Mamat, Z. C. & other authors (2010). Characterization of Nipah Virus from naturally infected *Pteropus vampyrus* bats, Malaysia. *Emerging Infectious Diseases* **16**, 1990-1993.

Randolph, S. E. & Dobson, A. D. (2012). Pangloss revisited: a critique of the dilution effect and the biodiversity-buffers-disease paradigm. *Parasitology* **139**, 847-863.

Ren, W., Li, W., Yu, M., Hao, P., Zhang, Y., Zhou, P., Zhang, S., Zhao, G., Zhong, Y. & other authors (2006). Full-length genome sequences of two SARS-like coronaviruses in horseshoe bats and genetic variation analysis. *The Journal of general virology* **87**, 3355-3359.

Reusken, C., Lina, P. H. C., Pielaat, A., de Vries, A., Dam-Deisz, C., Adema, J., Drexler, J. F., Drosten, C. & Kooi, E. A. (2010). Circulation of group 2 coronaviruses in a bat species common to urban areas in western Europe. *Vector-Borne and Zoonotic Diseases* **10**, 785-791.

Rihtaric, D., Hostnik, P., Steyer, A., Grom, J. & Toplak, I. (2010). Identification of SARS-like coronaviruses in horseshoe bats (*Rhinolophus hipposideros*) in Slovenia. *Archives Of Virology* **155**, 507-514.

Rostal, M. K., Olival, K. J., Loh, E. H. & Karesh, W. B. (2012). Wildlife: The Need to Better Understand the Linkages. *Curr Top Microbiol Immunol*.

Saif, L. J. (2004). Animal coronaviruses: what can they teach us about the severe acute respiratory syndrome? *Revue scientifique et technique* **23**, 643-660.

Salkeld, D. J., Padgett, K. A. & Jones, J. H. (2013). A meta-analysis suggesting that the relationship between biodiversity and risk of zoonotic pathogen transmission is idiosyncratic. *Ecol Lett* **16**, 679-686.

Sarukhán, J., Koleff, P., Carabias, J., Soberón, J. & Dirzo, R. (2009). Capital natural de México. Síntesis: conociiento actual, evaluación y perspectivas de sustentabilidad. México, D. F.: CONABIO.

Shirato, K., Maeda, K., Tsuda, S., Suzuki, K., Watanabe, S., Shimoda, H., Ueda, N., Iha, K., Taniguchi, S. & other authors (2012). Detection of bat coronaviruses from *Miniopterus fuliginosus* in Japan. *Virus Genes* **44**, 40-44.

Song, H. D., Tu, C. C., Zhang, G. W., Wang, S. Y., Zheng, K., Lei, L. C., Chen, Q. X., Gao, Y. W., Zhou, H. Q. & other authors (2005). Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. *Proceedings of the National Academy of Sciences of the United States of America* **102**, 2430-2435.

Suttle, C. A. (2007). Marine viruses--major players in the global ecosystem. *Nature reviews Microbiology* **5**, 801-812.

Suttle, C. A. (2009). Viruses: a vast reservoir of genetic diversity and driver of global processes. *Retrovirology* **6**.

Suzuki, J., Sato, R., Kobayashi, T., Aoi, T. & Harasawa, R. (2014). Group B betacoronavirus in rhinolophid bats, Japan. *The Journal of veterinary medical science / the Japanese Society of Veterinary Science* **76**, 1267-1269.

Tang, X. C., Zhang, J. X., Zhang, S. Y., Wang, P., Fan, X. H., Li, L. F., Li, G., Dong, B. Q., Liu, W. & other authors (2006). Prevalence and genetic diversity of coronaviruses in bats from China. *Journal Of Virology* **80**, 7481-7490.

Tao, Y., Tang, K., Shi, M., Conrardy, C., Li, K. S., Lau, S. K., Anderson, L. J. & Tong, S. (2012). Genomic characterization of seven distinct bat coronaviruses in Kenya. *Virus research* **167**, 67-73.

Taylor, L. H., Latham, S. M. & Woolhouse, M. E. (2001). Risk factors for human disease emergence. *Philosophical transactions of the Royal Society of London Series B, Biological sciences* **356**, 983-989.

Teeling, E. C., Springer, M. S., Madsen, O., Bates, P., O'Brien S, J. & Murphy, W. J. (2005). A molecular phylogeny for bats illuminates biogeography and the fossil record. *Science* **307**, 580-584.

Tong, S., Conrardy, C., Ruone, S., Kuzmin, I. V., Guo, X., Tao, Y., Niezgoda, M., Haynes, L., Agwanda, B. & other authors (2009). Detection of novel SARS-like and other coronaviruses in bats from Kenya. *Emerging infectious diseases* **15**, 482-485.

Towner, J. S., Pourrut, X., Albarino, C. G., Nkogue, C. N., Bird, B. H., Grard, G., Ksiazek, T. G., Gonzalez, J. P., Nichol, S. T. & other authors (2007). Marburg Virus infection detected in a common African bat. *PLoS ONE* **2**, 764.

Tsuda, S., Watanabe, S., Masangkay, J. S., Mizutani, T., Alviola, P., Ueda, N., Iha, K., Taniguchi, S., Fujii, H. & other authors (2012). Genomic and serological detection of bat coronavirus from bats in the Philippines. *Archives of virology* **157**, 2349-2355.

Turmelle, A. S. & Olival, K. J. (2009). Correlates of viral richness in bats (order Chiroptera). *Ecohealth* **6**, 522-539.

Van Der Hoek, L. (2007). Human coronaviruses: What do they cause? *Antiviral Therapy* **12**, 651-658.

van der Hoek, L., Pyrc, K., Jebbink, M. F., Vermeulen-Oost, W., Berkhout, R. J., Wolthers, K. C., Wertheim-van Dillen, P. M., Kaandorp, J., Spaargaren, J. & other authors (2004). Identification of a new human coronavirus. *Nature medicine* **10**, 368-373.

Vijaykrishna, D., Smith, G. J., Zhang, J. X., Peiris, J. S., Chen, H. & Guan, Y. (2007). Evolutionary insights into the ecology of coronaviruses. *Journal of virology* **81**, 4012-4020.

Wacharapluesadee, S., Sintunawa, C., Kaewpom, T., Khongnomnan, K., Olival, K. J., Epstein, J. H., Rodpan, A., Sangsri, P., Intarut, N. & other authors (2013). Group C betacoronavirus in bat guano fertilizer, Thailand. *Emerging infectious diseases* **19**, 1349-1351.

Watanabe, S., Masangkay, J. S., Nagata, N., Morikawa, S., Mizutani, T., Fukushi, S., Alviola, P., Omatsu, T., Ueda, N. & other authors (2010). Bat coronaviruses and experimental infection of bats, the Philippines. *Emerging Infectious Diseases* **16**, 1217-1223.

Wilson, D. E. & Reeder, D. M. (2005). *Mammal Species of the World*. Johns Hopkins University Press.

Wolfe, N. D. (2005). Bushmeat Hunting, Deforestation, and Prediction of Zoonotic Disease Emergence. *Emerging infectious diseases* **11**, 1822-1827.

Wolfe, N. D., Dunavan, C. P. & Diamond, J. (2007). Origins of major human infectious diseases. *Nature* **447**, 279-283.

Woo, P. C., Lau, S. K., Huang, Y. & Yuen, K. Y. (2009a). Coronavirus diversity, phylogeny and interspecies jumping. *Experimental biology and medicine* **234**, 1117-1127.

Woo, P. C., Lau, S. K., Yip, C. C., Huang, Y. & Yuen, K. Y. (2009b). More and more coronaviruses: Human Coronavirus HKU1. *Viruses* **1**, 57-71.

Woo, P. C., Lau, S. K., Chu, C. M., Chan, K. H., Tsoi, H. W., Huang, Y., Wong, B. H., Poon, R. W., Cai, J. J. & other authors (2005). Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *Journal of virology* **79**, 884-895.

Woo, P. C., Wang, M., Lau, S. K., Xu, H., Poon, R. W., Guo, R., Wong, B. H., Gao, K., Tsoi, H. W. & other authors (2007). Comparative analysis of twelve genomes of three novel group 2c and group 2d coronaviruses reveals unique group and subgroup features. *Journal of virology* **81**, 1574-1585.

Woo, P. C. Y., Lau, S. K. P., Li, K. S. M., Poon, R. W. S., Wong, B. H. L., Tsoi, H. W., Yip, B. C. K., Huang, Y., Chan, K. H. & other authors (2006). Molecular diversity of coronaviruses in bats. *Virology* **351**, 180-187.

Woo, P. C. Y., Lau, S. K. P., Lam, C. S. F., Lai, K. K. Y., Huang, Y., Lee, P., Luk, G. S. M., Dyrting, K. C., Chan, K. H. & other authors (2009c). Comparative analysis of complete genome sequences of three avian coronaviruses reveals a novel group 3c coronavirus. *Journal of Virology* **83**, 908-917.

Woo, P. C. Y., Lau, S. K. P., Lam, C. S. F., Lau, C. C. Y., Tsang, A. K. L., Lau, J. H. N., Bai, R., Teng, J. L. L., Tsang, C. C. C. & other authors (2012). Discovery of seven novel mammalian and avian coronaviruses in the genus *Deltacoronavirus* supports bat coronaviruses as the gene source of *Alphacoronavirus* and *Betacoronavirus* and avian coronaviruses as the gene source of *Gammacoronavirus* and *Deltacoronavirus*. *Journal of Virology* **86**, 3995-4008.

Wood, J. L., Leach, M., Waldman, L., Macgregor, H., Fooks, A. R., Jones, K. E., Restif, O., Dechmann, D., Hayman, D. T. & other authors (2012). A framework for the study of zoonotic disease emergence and its drivers: spillover of bat pathogens as a case study. *Philosophical transactions of the Royal Society of London Series B, Biological sciences* **367**, 2881-2892.

Woolhouse, M., Scott, F., Hudson, Z., Howey, R. & Chase-Topping, M. (2012). Human viruses: discovery and emergence. *Philosophical transactions of the Royal Society of London Series B, Biological sciences* **367**, 2864-2871.

Wu, Z., Ren, X., Yang, L., Hu, Y., Yang, J., He, G., Zhang, J., Dong, J., Sun, L. & other authors (2012). Virome analysis for identification of novel mammalian viruses in bat species from Chinese provinces. *Journal of virology* **86**, 10999-11012.

Xu, R. H., He, J. F., Evans, M. R., Peng, G. W., Field, H. E., Yu, D. W., Lee, C. K., Luo, H. M., Lin, W. S. & other authors (2004). Epidemiologic clues to SARS origin in China. *Emerging infectious diseases* **10**, 1030-1037.

Yang, L., Wu, Z., Ren, X., Yang, F., Zhang, J., He, G., Dong, J., Sun, L., Zhu, Y. & other authors (2014). MERS-related betacoronavirus in Vespertilio superans bats, China. *Emerging infectious diseases* **20**, 1260-1262.

Yang, L., Wu, Z., Ren, X., Yang, F., He, G., Zhang, J., Dong, J., Sun, L., Zhu, Y. & other authors (2013). Novel SARS-like Betacoronaviruses in Bats, China, 2011. *Emerging infectious diseases* **19**.

Yuan, J., Hon, C. C., Li, Y., Wang, D., Xu, G., Zhang, H., Zhou, P., Poon, L. L., Lam, T. T. & other authors (2010). Intraspecies diversity of SARS-like coronaviruses in Rhinolophus sinicus and its implications for the origin of SARS coronaviruses in humans. *The Journal of general virology* **91**, 1058-1062.

Yuen, K. Y., Lau, S. K. & Woo, P. C. (2012). Wild animal surveillance for coronavirus HKU1 and potential variants of other coronaviruses. *Hong Kong medical journal = Xianggang yi xue za zhi / Hong Kong Academy of Medicine* **18 Suppl 2**, 25-26.

Zaki, A. M., van Boheemen, S., Bestebroer, T. M., Osterhaus, A. D. & Fouchier, R. A. (2012). Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia. *N Engl J Med*.

Zepeda-Lopez, H. M., Perea-Araujo, L., Miliar-Garcia, A., Dominguez-Lopez, A., Xoconostle-Cazarez, B., Lara-Padilla, E., Ramirez Hernandez, J. A., Sevilla-Reyes, E., Orozco, M. E. & other authors (2010). Inside the outbreak of the 2009 influenza A (H1N1)v virus in Mexico. *PloS one* **5**, e13256.

Zhang, G., Cowled, C., Shi, Z., Huang, Z., Bishop-Lilly, K. A., Fang, X., Wynne, J. W., Xiong, Z., Baker, M. L. & other authors (2013). Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. *Science* **339**, 456-460.