Ecology, evolution, and spillover of coronaviruses from bats

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the third bat coronavirus to emerge and cause widespread human outbreaks in the past two decades. The significance of bats as coronavirus reservoir hosts was not recognized until the first SARS epidemic in 2002-2003. Since then, knowledge of bat coronaviruses has advanced rapidly, accruing over 4,000 coronavirus sequences from 14 bat families, yet the true diversity of bat coronaviruses is probably much greater. Given that bats are the likely evolutionary source for several human coronaviruses, including pathogens that cause the common cold, their role in historic and future pandemics requires rigorous investigation. We review and integrate the latest evidence on bat-coronavirus interactions at the molecular, tissue, host, and population levels that relate to spillover and pandemic risk. We identify critical knowledge gaps around the risk of coronavirus spillover to humans, including infection dynamics within natural host populations, the ability of bat coronaviruses to spread in human populations without prior adaptation in intermediate hosts, and genetic features that predict zoonotic capacity and pandemic potential. These and other gaps in knowledge of the ecology and evolution of coronaviruses constrain our ability to preempt emergence events and must be filled to prevent future pandemics.

Introduction

Bats are the main hosts of three of the ten zoonotic virus groups of pandemic concern, as designated by the World Health Organization: henipaviruses (Nipah, Hendra), filoviruses (Ebola, Marburg), and coronaviruses[1]. Common features among these emerging infections include their ability to cause severe disease in humans but not in the reservoir, their relatively rarely reported spillovers despite wide geographic distribution, and that they often rely on bridging hosts to amplify virus and increase opportunities for human infections. Spillover of multiple coronaviruses from bat to humans are consistent with an overall increasing number of emergent zoonotic infections from wildlife, mostly caused by human activities and exploitation of natural resources[2]. In particular, wildlife farming and trade facilitate cross-species transmission of viruses by mixing species in stressful and crowded conditions along supply chains[3,4]. The wildlife trade, and other human behaviors that promote contact with bat-borne pathogens such as hunting and guano mining, are part of larger patterns of encroachment into wildlife habitats and increasing pressure from human population expansion and intensifying natural resource use[5]. The COVID-19 pandemic has once again highlighted the need for integrated planetary health approaches to understanding spillover as a multilayered process. Here, we focus on the bat coronaviruses and the ecological, evolutionary, and epidemiological features that influence the risk of spillover into humans and their subsequent epidemic potential.

Bats are the second most diverse Order of mammals, with over 1,400 species, they host an exceptional diversity of coronaviruses with ancient viral lineages that are spread across all six continents that bats inhabit. Over 4,800 coronavirus sequences have been detected in bats, accounting for over 30% of all bat-viruses sequenced[6]. However, given that 524 bat species – out of a global diversity of 1,432 – have been sampled for coronaviruses (Supplementary Table 1), the true diversity of bat coronaviruses likely is much greater. Bats are hosts of ancestral lineages of betacoronaviruses from which viruses of public health concern, such as SARS-CoV, SARS-CoV-2, and Middle East respiratory syndrome (MERS-CoV) originate. The recent spillover events may just be the latest in a much longer history of spillover and emergence of bat-coronaviruses into humans. For example, of the four endemic human coronaviruses that cause 30% of common cold infections, two may have originated in bats (alphacoronaviruses HCoV-229E and HCoV-NL63)[7]. Thus, at least five of the seven coronaviruses capable of human-to-human transmission are directly linked to origins in bat coronaviruses[8,9]. The other two human coronaviruses also may have spilled over from animals to humans, the oldest from cattle approximately 100 years ago, which is also a betacoronavirus that might have spilled over from bats into cattle first[10]. Additionally, coronaviruses that infect other animal species also might have evolutionary origins in bats, such as the recently emerged coronavirus causing severe acute diarrhea syndrome (SADS-CoV) in pigs[11].

SARS-CoV-2 is the second emergent betacoronavirus of bat origin to cause widespread morbidity and mortality in humans in the past two decades. Its emergence was somewhat predictable given the prior examples of coronavirus transmission at the human-animal interface. Based on serological evidence of exposure of rural human populations to bat coronaviruses in China, spillovers from bats might occur more often than epidemics that are large enough to be detected by public health surveillance[12,13].

Here we review evidence about the ecology, evolution, and spillover of bat coronaviruses. We assess what is known about the determinants of coronavirus spillover and transmission among recipient hosts, from the geographic distribution of hosts and viruses through single virus-cell interactions. We further highlight the knowledge gaps that must be filled to inform preparation for and mitigation of coronavirus

emergence risk and suggest a research agenda for developing the science of preventing coronavirus spillover.

1 Distribution of bat coronaviruses

Coronaviruses (order *Nidovirales*, family *Coronaviridae*) include four genera: *Alphacoronavirus* and *Betacoronavirus*, which infect a broad range of mammals, and *Gammacoronavirus* and *Deltacoronavirus*, which primarily infect birds[14]. Since the emergence of SARS-CoV in 2002, and the evidence that it originated from a bat reservoir, coronaviruses have been detected in 16% (231) of bat species (Supplementary Table 1). Alphacoronaviruses and betacoronaviruses have been detected in bats from 14 of the 21 bat families, in at least 69 countries across six continents (Figures 1 & 2; Table 1; Supplementary Table 1). The diversity of coronaviruses found in bats is high, with over 60 coronavirus species (>4,000 individual sequences) detected from 13 of the 19 known mammalian *Alphacoronavirus* and *Betacoronavirus* subgenera (Figure 3). The apparent absence of coronaviruses in particular bat taxa is most likely due to insufficient sampling rather than true absence[15].

Sequence similarity among viruses in different hosts has been used to infer viral origins. Viruses with high sequence similarity to the three recently emerging human coronaviruses – SARS-CoV, SARS-CoV-2, MERS-CoV – have all been identified in bats (Figures 2 & 3). Coronaviruses from rhinolophid bats present up to 92% sequence identity to SARS-CoV[16] and up to 96% sequence identity to SARS-CoV-2[9] at the genome level. Additional SARS-related coronaviruses (SARSr-CoVs) have been detected in hipposiderid and molossid bats in Africa, Asia, and Europe (Supplementary Table 1), and it is now widely accepted that bats are the natural reservoir of SARSr-CoVs[17–19]. Similarly, although with lower sequence similarity, coronaviruses from vespertilionid bats show up to 86.5% sequence identity to MERS-CoV at the genome level[15]. MERS-related coronaviruses also circulate in bats within the families *Nycteridae, Emballonuridae*, and *Molossidae* in Africa, Europe, North America, and Asia (Supplementary Table 1), suggesting ancestral origin of MERS-CoV in bats, from which it spilled over onto dromedary camels (*Camelus dromedarius*), where along the course of its adaptation to its new host, MERS might have evolved zoonotic capacity[20]. The endemic human coronaviruses HCoV-229E (*Duvinacovirus*) and HCoV-NL63 (*Setracovirus*) have been detected in Africa and Southeast Asia in hipposiderid and rhinonycterid bats, respectively (Figures 2 & 3; Supplementary Table 1).

The wide distribution and high diversity of coronaviruses in bats is most likely the result of a long coevolutionary history. Some coronavirus groups seem to be exclusively associated with specific taxonomic groups of bats. For instance, the *Nobecovirus* subgenus has mostly been detected in Old World fruit bats (*Pteropodidae*); there is currently no evidence that nobecoviruses are zoonotic. Further understanding of the biogeography of bats and their coronaviruses will allow for identification of geographical areas of interest for surveillance and research that could contribute information on the bat-coronavirus dynamics from the individual to population levels.

1.1 Infection and response in bats

An important observation in emerging zoonotic viruses is that clinical signs of disease typically do not occur in reservoir host-virus infections even when humans might experience severe disease[21]. For example, SIV in chimpanzees, hantavirus in rodents, and Hendra and Nipah viruses in bats do not cause severe clinical signs[22]. In these systems, a long-term host-pathogen relationship has been established and the reservoir host often appears tolerant to the pathogenic effects of infection whereas the

147 spillover host develops severe tissue damage or strong immune responses with the associated
148 immunopathology[23]. Histopathological examination of bats experimentally infected with coronaviruses
149 demonstrated mild pathology, including rhinitis[24,25] and interstitial pneumonia[25]. In these studies, virus
150 or viral RNA was detected in the respiratory tract and/or intestines (e.g., Munster et al. 2016, Watanabe
151 et al. 2010). Whether all bat species are universally tolerant to coronavirus infection remains unclear as
152 a limited number of experimental coronavirus challenge studies of bats have been performed (Table 1,
153 Supplementary Information) and in most of these, the putative natural reservoir bat species was not
154 used, and infectious doses might not necessarily represent an average natural exposure. In this respect
155 the results from these experiments may not reflect the levels of tolerance and overall response to
156 infection in naturally infected individuals of the reservoir bat species.

157 While serological studies are generally used for surveillance of pathogens like Nipah virus, Marburg virus
158 and Ebola virus in bats, relatively limited information is available on serological investigation of
159 coronaviruses in bats. Some evidence suggests bat antibody responses to coronaviruses are relatively
160 weak and transient. In addition, there is even less data on coronavirus-specific innate immune response,
161 and it is unknown if first line innate defenses might render a robust antibody response less critical. For
162 example, seroconversion of bats after challenge with coronaviruses is not always observed[25,26]. In
163 experimental challenges of Egyptian fruit bats (*Rousettus aegyptiacus*) with WIV1-CoV (originally
164 isolated from a Chinese rufous horseshoe bat, *Rhinolophus sinicus*), no bats showed obvious signs of
165 disease and only two of 12 individuals seroconverted (measured by ELISA) although no neutralizing
166 antibodies were detected. In another experiment where Jamaican fruit bats (*Artibeus jamaicensis*) were
167 challenged with a human isolate of MERS-CoV, only one of the ten bats produced neutralizing
168 antibodies; in that study, pathological changes were detected in lungs and innate antiviral genes (Mx1,
169 Ccl5, and ISG-56) were modestly up-regulated[25]. These apparently poor antibody responses are likely
170 the result of the low capacity of the viruses to effectively infect the bat species used in the studies, or by
171 effective suppression of virus replication by the innate immune response. Both scenarios would result
172 in an absent or limited generation of a humoral response. This variability in adaptive humoral responses
173 in bats calls for caution in the interpretation of serological data. For instance, at the individual bat level,
174 inferences about previous infection might be limited and at the population level limited humoral
175 response coupled to rapid turnover might hamper interpretation on presence and absence of certain
176 pathogens. However, in-depth seroprevalence studies remain a useful guide to understanding the
177 epidemiological history of the population[22].

178 In bats, coronaviruses may have tropism for the respiratory and gastrointestinal tract. In the Jamaican
179 fruit bat study mentioned above, the highest loads of MERS-CoV RNA and infectious virus were detected
180 in the respiratory tract, with less virus in the intestines and internal organs[25]. Most recently, intranasal
181 inoculation of Egyptian fruit bats with SARS-CoV-2 resulted in transient respiratory infections, and the
182 highest viral loads were detected in the respiratory tract on day 4 post inoculation while oral and fecal
183 viral shedding was observed for up to 12 days[24]. Long periods of viral shedding in feces of three to 11
184 weeks have been reported in wild bats (*Myotis macropus*), supporting the importance of a potential
185 fecal-oral route of transmission; however, in that field study, potentially persistent infections could not
186 be distinguished from reinfections[27]. Viral RNA was also found in the intestines of Leschenault's
187 rousette (*Rousettus leschenaultii*) bats orally inoculated with a betacoronavirus isolated from a lesser
188 short-nosed fruit bat (*Cynopterus brachyotis*), but no infectious virus was isolated from recipient bats
189 nor was pathology observed, suggesting the species is not a competent host[28]. Further evidence of

190 tropism of coronaviruses for the gastrointestinal and respiratory system of bats comes from field studies
191 in which coronaviruses have been detected in intestines of little brown bats (*Myotis lucifugus*)[29], and
192 expression of the cell receptors used by multiple coronaviruses was high in both systems in fruit bats
193 while present only in the intestines of insectivorous bats[30].

194 In contrast to the limited number of experimental *in vivo* infections, a much greater number of
195 coronavirus infection studies have used bat cell lines (Table 1; Supplementary Information). Most of
196 these experiments focused on viral entry, providing insights into the ability of specific coronaviruses to
197 infect different species cell types. These experiments help make predictions of specific viruses' spillover
198 potential, though they provide limited insight into bat-virus interactions at the organismal level. Those
199 that make such inferences should be interpreted with caution. For example, two studies that used big
200 brown bat (*Eptesicus fuscus*) cells derived from the kidney, showed that innate antiviral genes,
201 specifically interferon beta, were not repressed by MERS-CoV[31]. Although long-term persistent MERS-
202 CoV infections was achieved in these big brown bat cells, inferences and conclusions about potentially
203 persistent infections in bats cannot be drawn without experimental infections of live bats to capture the
204 correct tissue tropism, physiological feedback that determines shedding, and thus, dynamics of infection
205 at the population level.

206 **1.2 Circulation in bat populations**

207 Prevalence of coronaviruses – estimated as the proportion of bats with detectable viral RNA in feces, or
208 fecal or oral swabs – presents high temporal and spatial variability (Figure 4). Overall, shedding of
209 coronaviruses tends to peak during summer or autumn in Australia and China[32–35], dry seasons in central
210 Africa and Asia[36], and wet seasons in Western or Southeastern Africa[7,37]. Although temporal trends differ
211 among studies, seasonal variations are consistently observed, pointing to potential mechanistic roles of
212 resource availability, reproductive cycles, and host behavior.

213 Although nutritional stress during periods of resource scarcity have been implicated in the shedding of
214 other bat viruses[38,39], their influence on coronavirus shedding is unclear, with effects differing by bat
215 species and virus variants. In Thailand, increased prevalence of both alpha- and betacoronaviruses was
216 associated with low body condition in Lyle's flying foxes (*Pteropus lylei*)[40]. In the Chinese rufous
217 horseshoe bat (*Rhinolophus sinicus*), low body condition was associated with increased shedding of one
218 variant of *Sarbecovirus* (SARSr-Rh-BatCoV), but not another (Rh-BatCoV HKU2)[41]. In Ghana, infection by
219 the alphacoronavirus Alpha229E-CoV correlated with low body condition in Noack's roundleaf bat
220 *(Hipposideros* cf. *ruber),* but not in the Aba roundleaf bat *(H. abae*)[37].

221 Colony size, density, and composition could also affect virus prevalence by changing transmission rates
222 both within roosts and between roosts. Roost composition affects viral circulation since multiple bat
223 species often roost together and viral competence will vary between host species and viral strains. For
224 example, mixed-species roosts in Yunnan Province, China, presented greater prevalence of SARSr-CoVs
225 when *Rhinolophus sinicus*, a primary host of SARSr-CoVs, was more abundant in the roost than other
226 species. In the same roost, the lowest prevalence was detected when *Aselliscus stoliczkanus* was the
227 most abundant bat species[34]. Roost size and location, including whether the roosts are in caves, seem to
228 affect the chance of spillover of viruses between host species – likely through close physical contact in
229 dense roosts[42]. In addition to heterogeneity in competence among host species, heterogeneity in
230 infectivity (e.g., superspreaders, aerosolization capacity) is a feature of coronavirus infections in

231 humans[43]. The extent to which this individual-level heterogeneity explains coronavirus transmission
232 dynamics in bats, the variation in prevalence among roosts, and the risk of spillover is unknown.

233 Reproductive cycles also influence prevalence and transmission of viruses in bat colonies by affecting
234 patterns of behavior and physiological susceptibility. Increased social contacts among Chinese
235 horseshoe bats during mating season and feeding after hibernation might explain peaks of SARSr-Rh-
236 BatCoV and Rh-BatCoV HKU2 infection observed in spring[41]. In species that form maternal roosts, for
237 example, increases in group size coincide with gestation, during which inflammatory immune responses
238 are downregulated, potentially facilitating infection and shedding[44,45]. Periparturient stress also seems
239 to affect viral shedding, as observed in greater horseshoe bats (*Rhinolophus ferrumequinum*), Geoffroy's
240 bats (*Myotis emarginatus*)[46], and mouse-eared bat (*Myotis myotis*)[47], in which both the proportion of
241 bats shedding virus and viral concentrations increased post-parturition. Similarly, in relation to
242 reproductive cycles, high prevalence and concentration of coronaviruses detected in Chinese horseshoe
243 bats (predominantly *Rhinolophus sinicus*) during September and October, when birth pulses take place,
244 are attributed to increases in the number of susceptible juveniles[32–34]. Additionally, cross-sectional
245 surveys of multiple bat species report higher infection rates or viral shedding in juveniles and subadults,
246 supporting age-related differences in susceptibility and competence of infection, consistently across
247 species[15,40,48,49]. Further field studies of multiple species across East Africa found that in both age
248 categories, shedding was highest during weaning[49] – timing that relates to behavioral changes,
249 physiological stress, and potential waning of maternal immunity.

250 Although the data exhibit some associations between seasonal factors and circulation of coronaviruses
251 in bats, the evidence is insufficient to predict dynamics of shedding. Many of the associations with
252 seasonal factors may be coincidental rather than causal and this could explain the lack of consistent
253 patterns across taxa and geographies. Small sample sizes and limited temporal resolution are common
254 issues in wildlife studies which hamper their statistical power. Coordinated and systematic approaches
255 to field studies that sample individual bats, paired with experimental inoculation and transmission
256 studies, and then integrated with modeling studies aimed at assessing the importance of factors driving
257 infection could vastly improve our understanding of coronavirus dynamics across species[50]. For example,
258 the extent to which seasonal factors drive other viral dynamics are dependent on the duration of
259 infection, transmission rate and the acquisition of protective immunity in bats[22] – variables that are yet
260 to be measured for any bat coronavirus. This evidence requires sampling with extensive replication in
261 time and space, alongside the collection of metadata[51].

262 **1.3 Co-infections in bats**

263 Co-infections of multiple pathogens can influence transmission to conspecifics and to spillover hosts.
264 While cross-protective immunity from concurrent or past infections might reduce susceptibility or
265 transmission of a pathogen, trade-offs in immune responses to co-infecting pathogens might facilitate
266 transmission[52]. Co-infection of bats with multiple coronaviruses at the same time, or co-circulation of
267 multiple virus genotypes within a roost, might result in interactions that affect timing, location, and
268 intensity of virus shedding, as has been described in other viral families[39]. As with other putative drivers,
269 the incidence and effects of coronavirus co-infections on viral transmission dynamics in bats have been
270 poorly studied. Co-infections between two coronavirus species[37,41,53–58] and between coronaviruses and
271 viruses from other families, including adenoviruses[59,60], astroviruses[59,61–63], herpesviruses[59], and
272 paramyxoviruses[64] have been described and are likely common. Cases of co-infections involving

273 coronaviruses range from 0.2% to 34.2% in wild bats[37,53–57] and as high as 73% in captive bats[65], and up
274 to 88% of virus-positive samples contained multiple viral families[61]. Frequent co-infection has potentially
275 important consequences because coronaviruses recombine frequently, providing an opportunity for the
276 emergence of new variants with enhanced spillover capacity.

277 Few studies have examined ecological interactions between co-infections of coronaviruses and non-viral
278 pathogens, including whether they are competitive, synergistic, or neutral. For instance, a 60-fold
279 increase in coronavirus (Myl-CoV) RNA was observed in the intestines of bats (*Myotis lucifugus*) co-
280 infected with the fungus that causes white nose syndrome (*Pseudogymnoascus destructans*)[66]. Systemic
281 down-regulation of anti-viral immune responses due to *P. destructans* infection was suggested as the
282 cause of increased coronavirus replication. Similarly, ectoparasite loads have been associated with
283 coronavirus infection; infection with Alpha229E-CoV almost doubled the risk of infection by BetaBI-CoV
284 in Noack's roundleaf bat but also correlated positively with loads of streblid flies, mites, and nycteribiid
285 flies[37]. Longitudinal studies tracking the health and immune status of individual bats, including co-
286 infections, are crucial to understanding the dynamics of bat viruses.

287 2 Molecular evolution and host range

288 2.1 Viral genetic diversity and evolution

289 Coronaviruses possess the largest genome among the RNA viruses, allowing for potentially rapid
290 evolution via mutation and recombination[67]. In general, RNA viruses have higher mutation rates than
291 DNA viruses, while also larger genomes provide more opportunities for mutations to occur.
292 Approximately two-thirds of the coronavirus genome encodes an RNA-dependent polymerase (RdRp)
293 and other non-structural proteins. The remaining third encodes four structural proteins – spike,
294 envelope, membrane, and nucleocapsid – and accessory proteins[68]. The genomes of coronaviruses
295 replicate via the RdRp enzyme, which is generally error-prone during replication, resulting in mutations
296 at significantly higher frequency than seen in other viruses[69,70]. However, the three largest viral families
297 in the order *Nidovirales* – *Coronaviridae*, *Roniviridae*, and *Mesoniviridae* – all encode a 3'-5'
298 exoribonuclease that improves their RNA replication fidelity, reducing the chances of mutations that can
299 reduce viral fitness[71–74]. The activity of the exoribonuclease might vary in different hosts, allowing for
300 modulation of viral evolution and therefore spillover capacity, with different host species presenting
301 heterogeneities in their competence as "superevolver" hosts that may influence the emergence of new
302 variants[15,19,75–77].

303 Recombination of large coronavirus genomes is common, which creates additional genetic diversity,
304 further expanding viral evolution and potential for shifts in cell tropism and host range[78]. Coronaviruses
305 replicate in the cytoplasm, where subgenomic RNAs are generated, facilitating recombination of genes
306 from different coronavirus lineages that might co-infect a host cell. This is also a result of the RdRp.
307 During genome replication, the RdRp presents a random "copy-choice" mechanism, in which the
308 enzyme "jumps" from one RNA template molecule to another one that may come from different viral
309 genomes[78,79]. These recombination processes have been implicated in the emergence of numerous
310 novel coronaviruses, such as murine coronavirus [80], transmissible gastroenteritis virus[81], feline and
311 canine coronaviruses[82,83], and in human OC43[84], NL63[85], HKU1[86], SARS-CoV[87,88], and MERS-CoV[89].
312 Interestingly, recombination of coronavirus genomes not only occurs among coronaviruses, but also
313 between coronaviruses and other families of RNA viruses including those from *Astroviridae* and

314 *Reoviridae*[90], resulting in an even greater potential for evolution, and therefore the emergence of
315 spillover and zoonotic potential when ecological conditions are favorable.

316 Mutation, recombination and host competence for infection and co-infection might have facilitated the
317 extraordinary diversity of coronaviruses, diversity particularly seen in bats[15,91]. Some families of bats
318 appear to have coevolved over millions of years with particular subgroups of betacoronaviruses:
319 rhinolophid bats and the SARS-related sarbecoviruses, vespertillionid bats and the MERS-related
320 merbecoviruses, and pteropodid bats and nobecoviruses, which have not been implicated in zoonosis[91].
321 Host-switching, resulting from successful broad jumps in host range, are most common in the
322 rhinopholid–*Sarbecovirus* clade[15,19,92]. Altogether, the high evolutionary potential of bat coronaviruses
323 directly influence host and tissue tropism, host range, transmissibility, and infection severity in new
324 hosts. Indeed, once established in a new host population, evolution might give rise to more fit lineages
325 exhibiting higher transmissibility, as observed for SARS-CoV-2 in humans[93].

326 **2.2 Host receptor recognition**

327 In coronaviruses, the capacity of the virus to enter a host cell is mediated by the spike protein, which
328 supports both binding to the host cell – through its receptor binding domain (RBD) – and the fusion with
329 its membrane[68]. The RBD attaches to host-cell receptors, which are membrane proteins or sialic acids.
330 For example, HCoV-NL63, SARS-CoV, and SARS-CoV-2 bind angiotensin-converting enzyme 2 (ACE2),
331 MERS-CoV binds dipeptidyl peptidase 4 (DPP4), and HCoV-229E, canine coronavirus (CCoV), and several
332 porcine and feline coronaviruses bind alanine aminopeptidase (APN), while HCoV-OC43, HCoV-HKU1,
333 and bovine coronavirus (BCoV) bind N-acetyl-9-O-acetylneuraminic acid[94–97].

334 The interaction between the RBD of the coronavirus spike and the host receptor can be thought of as a
335 match between a key and a lock. Thus, the specific structures of both the virus RBD and the receptors
336 available on a potential host determine in large part the host range of a coronavirus. While natural
337 selection would favor host-virus interactions with perfect matches, RBDs with a structure "close
338 enough" to a perfect match could still bind host receptors. The wide host range of several coronaviruses
339 can be explained by the fact that some cell receptors are highly conserved across animal species, such is
340 the case of ACE2, DPP4 and APN[98,99]. However, small differences in receptor structure across potential
341 hosts, such as glycosylation profile or single amino acid changes, can moderate receptor affinity[94].
342 MERS-CoV spike, for instance, binds the DPP4 of various species of primates, hooved mammals and bats,
343 but not of ferrets and rodents; this difference is explained by changes in five amino acids in the host
344 receptor[98]. Thus, direct coronavirus spillover from bats might be facilitated by similarities of the cell
345 receptor structures and RBD identity of both species. This is a critical aspect for characterization of
346 zoonotic potential of extant bat coronaviruses, however there is scarce knowledge of what receptors
347 different coronaviruses use in their reservoir bat hosts. Very few studies have been conducted on
348 receptor profiles of bats. It is currently known that several bat coronaviruses bind to ACE2 and DPP4,
349 however structural modeling and biochemical data (receptor binding assays and/or pseudotyped viral
350 entry assays) support differing degrees of binding affinity[98–100] and therefore potential for successful
351 infection of human cells. This variation in binding affinity is driven by differences at the level of single
352 amino acid residues interacting between the spike RBD and the receptor, suggesting that zoonotic
353 capacity could emerge in a few evolutionary steps.

354 The zoonotic potential of most bat coronaviruses is unknown, in part because isolates are difficult to
355 obtain, and therefore most inferences are based on genomic sequences. There is a need for high

356 throughput analyses of sequences and carefully controlled cell-line experiments to assess spillover and
357 zoonotic potential of the extant coronaviruses variants currently circulating in bats[1]. *In silico* analysis of
358 ACE2 and other receptors of human and other species are useful for initial identification of susceptible
359 species that could serve as bridge or reservoir hosts of zoonotic coronaviruses, with important value for
360 optimization of resources for preemptive surveillance. For instance, relatively conserved SARS-CoV-2
361 binding residues have been reported across the ACE2 sequences of non-human primates, hooved
362 mammals, felids, and cetaceans, suggesting potential susceptibility to infection[101]. Several of these
363 predictions have been validated by empirical studies confirming the broad host range of SARS-CoV-
364 2[99,102]. However, these studies also classified horseshoe bats, pangolins, minks, and ferrets as unlikely
365 hosts of SARS-CoV-2, yet field and laboratory data have reported their susceptibility to SARS-CoV-2 or
366 related viruses, highlighting the need for empirical validation of model predictions[102,103].

367 Contrasting results between *in silico* and empirical studies could emerge from different receptor use
368 among virus species in different hosts. Multiple studies suggest that the progenitor viruses of SARS-CoV
369 and SARS-CoV-2 may not utilize the ACE2 receptor in their original bat hosts [101,104,105]. However, this
370 could also be the result of variability in the host receptors with which the viruses have evolved, favoring
371 specific interactions between RBD and small residues of the host receptors. Thus, those progenitor
372 viruses may be adapted to their specific reservoir ACE2, but not to human ACE2[100], on which many *in*
373 *silico* and empirical studies are based[101]. Further variation in results and predictions can result from the
374 natural and high variation of the ACE2 receptor among bat species[106] in conjunction with the high
375 diversity of SARSr-CoVs[107]. Expanding considerations of virus-receptor pairs would shed more light on
376 receptor usage by SARSr-CoVs in bats. In any case, mutations were likely necessary in the spike of those
377 progenitors, and potentially selection in a bridge host, to allow the evolution of binding capacity to
378 human ACE2 and facilitate zoonotic spillover[105,108]. Such a case is supported by the use of human DPP4
379 receptor by MERS-CoV, affinity that may have emerged as a result of the evolution of the virus in
380 dromedary camels, after the initial spillover from bats[77].

381 Once a coronavirus RBD presents affinity for a receptor on a host cell, the heterogeneous distribution of
382 those receptors in different cell types within a host will influence tissue tropism, impacting pathogeny
383 and transmission. In humans, ACE2 is expressed primarily in epithelial cells of many tissues, including the
384 respiratory airways, kidney, heart, and digestive tract, consistent with the respiratory and
385 gastrointestinal pathology of SARS-CoV and the multisystemic pathology of SARS-CoV-2[109,110]. Although
386 detailed expression profiles of ACE2 in other species are lacking, tissue tropism of SARSr-CoVs is
387 consistent with humans. SARSr-CoVs have been detected in the respiratory or gastrointestinal tracts of
388 Malayan pangolins (*Manis javanica*)[111], experimentally inoculated ferrets, felids[102,112], and non-human
389 primates[113]. Similarly, DPP4 expression in humans, dromedary camels, and fruit bats include epithelial
390 cells of the respiratory and gastrointestinal tracts, with variability across species[30]. However, in humans,
391 while ACE2 expression levels are particularly high in the upper respiratory tract, DPP4 is mainly
392 expressed in the lower respiratory tract, which could contribute to the greater human-to-human
393 transmissibility of SARS-CoV and SARS-CoV-2 compared to MERS-CoV[30,114]. In addition, DPP4 expression
394 is almost entirely restricted to intestines in two vespertilionid bats, the suggested reservoir of the MERS-
395 CoV progenitor, suggesting different tropism, and potentially transmission routes, between reservoir
396 and spillover hosts[30]. Nevertheless, the detection of coronaviruses in the respiratory and gastrointestinal
397 tracts of experimentally inoculated and wild-caught bats supports the relevance of these two systems
398 for coronavirus infections among diverse host species[17,25,29,41,79,91].

399 ### 2.3 Host proteases and host range

400 Besides binding to the cellular receptor, successful infection and replication require several consecutive
401 steps, including entry, replication, potential evasion of the host specific innate immune response and
402 budding. In addition to the spike protein, host proteases are key to determining host range and tissue
403 tropism, which are needed to activate (cleave) the virus spike to allow entry to host cells. This spike-
404 protease interaction might be as important as host receptor binding in determining its zoonotic
405 potential[97,115] and potentially human-to-human transmissibility[93]. Spike proteins of coronaviruses have
406 multiple cleavage sites that interact with host proteases at different stages of the cell infection cycle[114].
407 Transmembrane serine proteases (e.g., TMPRSS2), trypsin-like proteases, and other cell-surface
408 proteases participate in spike cleavage after viral attachment, while lysosomal proteases such as
409 cathepsins intervene after virus endocytosis in target cells. In contrast, the furin proprotein convertase –
410 present in the Golgi apparatus – is involved in spike cleavage during biosynthesis in virus-producing cells
411 [116,117]. The distribution and activity of these proteases vary among cell types and physiological
412 conditions, therefore influencing tissue tropism and cell entry[114,118,119]. For instance, the respiratory
413 tropism of SARS-CoV might be driven by trypsin-like proteases present in respiratory cells[120,121].

414 Therefore, the expression patterns of proteases also directly contribute to host range. For instance,
415 whereas specific bat proteases cleave the spike proteins of both MERS-CoV and bat HKU4-CoV, allowing
416 entry into host cells, human proteases cleave only the MERS-CoV spike proteins[122]. The ability of
417 coronavirus spikes to adapt to being activated by proteases of new hosts (e.g., type, activity, and
418 distribution) after spillover is not well understood, but is essential to predicting the potential for
419 changes in host range and tissue tropism, including spillback (i.e., infection of the origin host via the
420 recipient host) and reverse zoonotic transmission.

421 ## 3 Exposure and spillover into humans

422 The great diversity of bat species in which alpha- and betacoronaviruses have evolved, in conjunction
423 with the intrinsic evolutionary adaptability of these RNA viruses are important drivers of the chances of
424 zoonotic capacity naturally emerging among coronaviruses circulating in bats. However, for zoonotic
425 spillovers to occur, humans must be exposed to coronaviruses capable of spillover (Box 1). This exposure
426 can occur through direct contact with virus excreted from an infected bat or bridge host, or through
427 other contacts with the infected animal such as slaughter or butchering. The nature and intensity of the
428 reservoir bat-human interface is critical to determining the risk of spillover. In both situations, one
429 primary determinant of exposure is human behavior that increases contact with bats, or behaviors of
430 other animals that may bridge the contact between infected bats and susceptible humans. Very little is
431 known about the specific conditions of coronavirus spillovers, but we can propose a few plausible
432 hypotheses. Some human behaviors that may contribute to coronavirus spillover include activities such
433 as bat hunting and consumption, guano farming, and wildlife trading[3,123]. These activities could pose a
434 risk due to close contact between humans and bats under physiologically stressful situations that may
435 increase viral shedding, and therefore the infectious doses received by potential human "patients zero".
436 These activities often occur in rural areas with limited access to healthcare, reducing the detectability of
437 spillover events to only cases that may trigger local or regional epidemics. The factors that led to
438 outbreaks of some of the recently emerged human coronaviruses are known, such as the roles of bridge
439 hosts in the wildlife trade and among domestic animals, however their relative importance in the
440 process is not very clear. In particular, SARS-CoV likely spilled over from rhinolophid bats into humans

441 via farmed Himalayan civets (*Paguma larvata*)[79,124,125]. Alternative pathways of direct human exposure to
442 bat coronaviruses have not been explored thoroughly. Characterizing the bat-human interface through
443 studies that specifically target human populations at risk such as guano farmers, bat hunters, and
444 wildlife traders for evidence of bat coronavirus exposure[126], and the role of other species in the
445 transmission chain, remains crucial to effective surveillance, response, and prevention of future zoonotic
446 coronavirus pandemics.

447 **3.1 Reservoir animal-human interface**

448 Human-bat interactions vary widely in space, time, nature, and intensity, with some of the species of
449 bats rarely encountering humans, while others have frequent contact. For example, humans in Oceania,
450 Asia, Africa, South America and Pacific islands have long hunted fruit bats for food[127,128]. Harvested
451 guano from bat caves, which could harbor infectious virus, has provided agricultural fertilizer in
452 Thailand, Philippines, Indonesia, Mexico and the United States[129]. The long-term bat-human interactions
453 contrast with the apparently contemporary and increasing emergence of highly virulent infections in
454 humans linked to bats. Land-use change, animal domestication, and human expansion into wildlands,
455 among other factors, have been linked to the emergence of infectious diseases in general, and most
456 likely play a role in the spillover of bat-borne viruses[5]. Changes in the quality of bats' habitat may also
457 affect their overall health and the circulation of viruses due to factors such as stress[130]. Low food
458 availability, mediated by climate and deforestation, is a hypothesized driver of viral shedding in bats,
459 including the zoonotic Hendra and Nipah virus[131,132]. In support of this hypothesis, coronavirus shedding
460 in horseshoe bats was reported to be higher in human-dominated than in natural landscapes[36]**.** In
461 addition, the increasing diversity of species ending up in the legal and illegal wildlife trades results in
462 viruses being transported over longer distances and maintained in unsanitary conditions, likely
463 increasing transmission, as demonstrated by coronaviruses in rodents[3] and MERS-CoV in dromedary
464 camels[133].

465 **3.2 Direct bat-to-human spillover**

466 There are currently no recorded cases of transmission of coronaviruses directly from bats to humans.
467 However, there are good reasons to suspect surveillance biases rather than a genuine absence of
468 spillovers. If spillovers occur in rural areas or in low-income countries where human-bat contacts are
469 common but access to health care is limited, they may go undetected. Furthermore, infection by some
470 bat coronaviruses might be asymptomatic in humans or cause symptoms that are mistaken for common
471 diseases. Even for highly virulent pathogens where surveillance programs exist, such as Ebola virus or
472 Nipah virus, reported spillover events are only the tip of the iceberg[134,135] and only get recognized after
473 significant human-to-human transmission. In the case of Ebola virus, it takes on average 44 days of
474 undetected transmission between primary and index cases before an outbreak is recognized[136].

475 Bat coronaviruses face numerous barriers to infect and spread among humans. Barriers occur at the
476 level of exposure (lack of bat-virus-human contact), infection (coronavirus is not compatible with
477 humans), or transmission (virus cannot efficiently transmit among humans). Thus, only a minority of
478 human exposures may lead to infection, and even fewer to onward transmission. Studies in Asia have
479 found serological evidence of human exposure to SARS-CoV or related viruses in healthy adults in Hong
480 Kong and army recruits on the mainland China sampled prior to the 2002 SARS pandemic[137,138]. More
481 recent studies of villagers in the southern Chinese province of Yunnan found low seroprevalence of
482 antibodies to SARS-related coronaviruses[12,13]. These studies suggest that numerous coronaviruses are

483 breaching the exposure and infection barriers, although the low seroprevalence (<3%) indicates that
484 these spillovers are rare, and these viruses are not efficiently spreading in the human population. It is
485 unknown whether the observed seroprevalence arises entirely from primary spillover cases, or from a
486 combination of primary cases with limited human-to-human chains of transmission[139,140]. Additional
487 syndromic surveillance at health care facilities, combined with improved unbiased molecular diagnostic
488 tools that could target unknown pathogens, and periodic serologic surveys of human populations are
489 important tools to provide better understanding when, where, and how coronavirus spillovers from bats
490 occur.

491 **3.3 Spillover through bridging hosts**

492 Other animal species may provide an ecological, amplifying or evolutionary 'bridge' for coronavirus
493 transmission from bats to humans[8,79]. Bridging hosts may have high exposure to both bats and humans,
494 or they may produce high viral loads. This will lead to a higher probability of human exposure to both
495 the virus as well as a sufficiently high infectious dose, such as with horses and Hendra virus in Australia,
496 where the initial spillover and infection of the horses leads to exposure and infection in humans[141].
497 Alternatively, evolutionary bridging hosts may allow genetic adaptation of the virus, resulting in new or
498 enhanced zoonotic capacity, such as the case of MERS-CoV in dromedary camels[79].
499
500 Putatively, farmed Himalayan palm civets served as bridge hosts in the spillover of SARS-CoV from bats
501 to humans, and selection for enhanced viral replication in civets may have favored viral mutations that
502 increased zoonotic capacity[79,124,125]. In the case of the human coronavirus HCoV-OC43, molecular clock
503 analysis indicates that a rodent betacoronavirus (*Rattus* spp. coronavirus HKU24) spilled over to bovines
504 thousands or millions of years ago, followed by a second spillover from bovines to humans during the
505 last few hundred years[142–144]. Thus, new spillover hosts (as bovines in the case of ancestral HCoV-OC43)
506 might allow for efficient viral replication and transmission favoring viral evolution. Similarly, endemic
507 circulation of MERS-CoV in dromedary camels suggests that transmission of ancestral merbecoviruses
508 from bats to camelids occurred decades or much longer ago, giving rise to the emergence and
509 establishment of MERS-CoV in camelids and likely resulting in the evolution of capacity to infect
510 humans[133,145]. Thus, MERS-CoV is considered a camelid virus with ancestral origins in bats[146–148].

511 The ecological and evolutionary conditions that facilitated the recent spillover of SARS-CoV-2 remain
512 unknown for now. Whether the first transmission event of SARS-CoV-2 happened directly from bats to
513 humans, possibly via bat feces, or through a bridging host in which a bat-derived ancestor of SARS-CoV-2
514 evolved the ability to infect humans (eco-evolutionary process) are two plausible hypotheses at the time
515 of writing. Coronaviruses closely related to SARS-CoV-2, which may have the capacity to infect humans,
516 appear to have circulated in bats for decades, supporting the possibility of direct bat-human
517 transmission[107].

518 In addition, humans can act as bridging hosts that could cause reverse zoonosis of SARS-CoV-2 to other
519 animals, including domestic cats, dogs, large felids (e.g., tiger [*Panthera tigris*]), and farmed American
520 minks (*Neovison vison*), which can become infected and then act as reservoirs for new variants. In the
521 specific case of farmed minks, SARS-CoV-2 can spread at epidemic levels, facilitating adaptation of the
522 virus to the new host[149]. Thus, spillback to other wildlife species could potentially lead to establishment
523 in secondary reservoirs. ACE2 sequences of Cricetidae rodents suggest many are putatively susceptible
524 to SARS-CoV-2. Old World Syrian hamsters (*Mesocricetus auratus*), Chinese hamsters (*Cricetulus*

525 *griseus*), and New World North American deer mice (*Peromyscus maniculatus*) are cricetid rodents that
526 are susceptible to SARS-CoV-2[150–153]. Whereas a large variety of wild and domestic animal species are
527 susceptible and could even transmit the virus amongst themselves (e.g. [149,151,154,155156]), it is unclear for
528 these species how transmission dynamics, population size, structure and connectivity, and eventual
529 herd (cross) immunity would allow for the establishment of continuous reservoirs or just temporary
530 reservoirs. This evidence for potential reverse zoonosis or spillback from humans to animals calls for
531 further research to elucidate the potential for new wild species to established as new viral reservoirs.

532 4 Knowledge gaps and research agenda

533 Fundamental knowledge gaps remain about the multilayered conditions that result in coronaviruses
534 flowing from bats into humans. Dynamic integration among field studies, modelling, laboratory
535 experiments, and human epidemiology can provide the insights required to prevent new coronavirus
536 spillover and pandemics.

537 The extensive study of coronavirus diversity in wild bats has yet to translate into a more profound
538 understanding of their zoonotic capacity. For instance, it is unknown whether coronaviruses circulating
539 in bat populations can be transmitted directly to humans and if they can transmit among humans with R
540 > 1 without first adapting in a bridging host species. Combining the probabilistic ecological drivers of
541 spillover with a molecular mechanistic basis will lead to a more comprehensive understanding of the
542 zoonotic capacity of coronaviruses at large. Mechanistically, high throughput characterization of the
543 zoonotic potential of coronaviruses using a tiered system of *in silico*, *in vitro*, and *in vivo* methods is
544 needed to understand the potential risk to humans at a scale that is commensurate with the distribution
545 of risk.

546 Despite the rapidly growing number of genomic sequences of bat coronaviruses, our knowledge of their
547 ecology and evolution is incipient. Identification of how, when, and where viral shedding takes place will
548 directly inform the temporal and spatial layers of spillover risk assessment determined by higher
549 pathogen pressure, the first step in the spillover cascade. It remains unclear whether the temporal and
550 spatial patterns of coronavirus prevalence and intensity of shedding in bats are generalizable across
551 systems. To fill this gap, we need longitudinal studies at multiple scales of replication, from the
552 individual level to the population and metapopulation levels. These individual and population-level
553 studies, coupled with phylodynamic analyses of virus populations, will help to understand the natural
554 drift and evolution of bat-coronavirus variants that may result in emergence of cross-species and
555 zoonotic capacity in the reservoir host.

556 Our current predictive capacity of spillover is compromised by the apparent rarity of spillover events
557 and the limited ecological data available for them. Realistic assessment of spillover risk requires an
558 increased capacity to detect these events, especially when they do not trigger epidemics and are missed
559 by public health surveillance systems. Serosurveys in human populations at risk of exposure to bat-
560 coronaviruses need to be prioritized. Development of multiplex serological technologies, such as
561 Luminex or Virscan, can facilitate wide screening, even when the etiological agent has not been fully
562 characterized. Coupling this human-focused surveillance with spatial and temporal information of bat-
563 virus interactions, viral discovery and functional characterization are needed to estimate the magnitude
564 and frequency of spillover events that might have gone undetected in the past, thereby enhancing our
565 predictive ability for future events. It is urgent to implement this field research agenda, targeting high-

risk interfaces, particularly areas of fast environmental changes with human encroachment into wild habitats, wildlife trade supply chains, and farming areas with extensive wildlife contact.

Finally, as we fill the gaps and integrate knowledge across scales and disciplines, we can develop proactive strategies for spillover prevention, rather than rely on reactive outbreak mitigation. The exponential nature of epidemic growth makes stopping a new pathogen with efficient person-to-person transmission a difficult task, as demonstrated by SARS-CoV-2. As we understand the conditions that facilitate spillover, interventions to prevent those conditions will become clearer, opening opportunities for proactive actions to prevent the start of the spillover cascade that could trigger a new coronavirus pandemic.

5 Conclusion

Coronaviruses linked to bat origins have spilled over into humans multiple times, and most likely will continue to be a threat to public health in the future. The diversity of bats and the features of bat-coronavirus systems, from the scales of molecules to landscapes, play an important role in facilitating the natural evolution of zoonotic capacity. However, these pathogens cannot cause outbreaks in humans unless the requirements of spillover and onward transmission are met. Eventual risk of spillover depends on the characteristics of the human-animal interface, which is increasingly influenced by habitat deterioration and encroachment into wild areas. Integration of ecological, evolutionary, and epidemiological data from bat-virus systems, coupled with human epidemiological and health surveillance in high-risk areas, and the development of new tools for detection of spillover events are urgent for improving risk assessment and predictive capacity. This integration of scientific fields will provide the basis for a paradigm shift from mitigating coronavirus outbreaks to prevention of spillover of coronaviruses into humans in first place.

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7 Author contributions

M.R.-A., C.M., and R.K.P. conceived the study. M.R.-A., C.M., R.K.P., A.G., C.R.P., E.S.G., J.O.L.-S., P.H., and V.J.M contributed substantially to discussion of the content. M.R.-A., C.M., C.F., E.J., L.D., D.J., and M.K. compiled the data and performed formal analysis. M.R.-A, and C.M. contributed equally. All authors wrote the article. All authors reviewed and/or edited the manuscript before submission.

601 ## 8 Competing interests

602 We declare no competing interests.

603 ## 9 Figure legends and tables

604 **Table 1.** Summary of 203 original studies on coronaviruses in bats. Study types were not exclusive, so a
605 study may fit into multiple types depending on the sampling approach and analytical methods. More
606 details are provided in Supplementary Table 2 and all classified studies can be found in Supplementary
607 Dataset 1.

Study type	Number of studies	Overview
Experimental	Bat cell lines: 28 Live bats: 6	Bat cell experiments • Target cells: brain, embryo, intestine, kidney, lung • Tested viruses: multiple bat SARS-like CoVs, BatCoV HKU4, BatCoV HKU9, HCoV-229E, HCoV-NL63, MERS-CoV, PEDV, Ro-BatCoV GCCDC1, SADS-CoV, SARS-CoV, SARS-CoV-2, *Scotophilus* bat CoV 512, TGEV Live bat experiments • Tested hosts and viruses: *Artibeus jamaicensis* (MERS-CoV), *Eptesicus fuscus* (SARS-CoV-2), *Myotis lucifugus* (Myl-CoV), *Rousettus leschenaultii* (BatCoV HKU9), *Rousettus aegyptiacus* (bat SL-CoV WIV1, SARS-CoV-2)
Longitudinal	14	• Countries: Australia, China, Denmark, Germany, Malaysia, Singapore, South Korea, Thailand (n = 8) • Serially sampled bat families: Pteropodidae, Hipposideridae, Vespertillionidae, Rhinolophidae (n = 4) • Serially sampled species: *Eonycteris spelaea*, *Hipposideros cervinus*, *Myotis daubentonii*, *Myotis macropus*, *Myotis myotis*, *Pteropus lylei*, *Rhinolophus sinicus*, *Rousettus leschenaultii* (n = 8)
Surveys	Cross-sectional (intra-species): 12 Cross-sectional (inter-species): 116 CoV detection and sequencing only: 28 Multi-pathogen detection: 33	• Sampled countries: primarily in Asia, Africa, and Europe; fewer in the Americas or Oceania (n = 69) • Sampled bat families: all bat families have been sampled at least once except Cistugidae, Furipteridae, and Myzopodidae (n = 18) • Positive bat families: 14 • Sampled bat species: 524 • Positive bat species: 231

608

609 **Figure 1. Geographic and taxonomic distribution of reported bat hosts of coronaviruses.** (A)
610 Biogeographic patterns of bat families, sampling, and coronavirus host status. (B) Bat taxonomic
611 diversity and coronavirus testing results. Data were compiled from field studies involving sequencing of
612 coronaviruses in wild bats. A list of all reported bat coronavirus hosts based on the reviewed studies can
613 be found in Supplementary Table 1 and Supplementary Dataset 1. The number of bat species per family
614 is based on the expert-curated Bat Species of the World database (https://batnames.org/).

615 **Figure 2. Geographic distribution of reported bat hosts of coronaviruses.** Data on bat hosts were
616 compiled from field studies involving sequencing of coronaviruses in wild bats. Where phylogenetic
617 analysis was included in studies, key coronavirus subgenera of bats associated with human or domestic

618 animal disease were summarized (see Supplementary Dataset 1). Geographic ranges of reported bat
619 host species for any coronaviruses or key subgenera were retrieved from IUCN. Plots display the number
620 of bat species based on overlapping geographic ranges. The plot of bat species includes 1,306 species
621 with IUCN range data.

622 **Figure 3. Coronavirus taxonomy and host distribution.** The proposed phylogeny has been compiled
623 from analyses of full genomes and/or gene segments. Branch lengths do not reflect evolutionary
624 distance between taxa and are drawn only to clearly illustrate relationships between and within genera.
625 The distribution of bat species hosting highlighted subgenera are represented in Figure 3. The associated
626 table summarizes a selection of important pathogenic virus species within genera and the host species
627 or taxa with reported infections of a virus within a genus. BCoV – bovine coronavirus, CCoV – canine
628 coronavirus, CRCoV – canine respiratory coronavirus, FCoV – feline coronavirus, IBV – avian coronavirus
629 (infectious bronchitis virus), HCoV – human coronavirus, MCoV – murine coronavirus, MERS – Middle
630 East respiratory syndrome, Myl-CoV – *Myotis lucifugus* coronavirus, PEDV – porcine epidemic diarrhea
631 virus, PorCoV – porcine coronavirus, SADS-CoV – swine acute diarrhea syndrome coronavirus, SARS –
632 severe acute respiratory syndrome, TGEV – transmissible gastroenteritis virus.

633 **Figure 4**. **Prevalence of detection of bat coronaviruses in field samples.** Data obtained from published
634 studies including two or more sampling events with at least 10 samples and reported positive and
635 negative samples. While the data show that prevalence varies in space and time and by bat species
636 (each plot), few studies provide insights on its drivers. No field studies combine longitudinal sampling of
637 individuals with collection of extensive metadata on bat ecology and condition. Sampling designs vary
638 across studies, with most of the studies conducting cross-sectional sampling over multiple years. One
639 field study sampled individual bats at multiple sites over time, although they pool data across three
640 sites[40] panel A. Other studies sampled the same bat species over time across multiple sites or sampled
641 multiple species and individuals in pooled samples across time within a site. These sampling approaches
642 reflect the purpose of the studies—most focused on characterizing viral diversity, not infection
643 dynamics. Details presented in Supplementary information. Each plot represents the prevalence of
644 detections per bat species. A) *Pteropus lylei*[40], B) *Eonycteris spelaea*[65], C) *Rousettus leschenaulti*[157], D)
645 *Chaerephon pumilus*[49], E) *Eidolon helvun*[49], F) *Lissonycteris angolensis*[49], G) *Rhinolophus cf. clivosus*[49], H)
646 *Myotis daubentoniid*[158], I) *Rhinolophus sinicus*[159], J) *Rhinolophus sinicus - Rhinolophus ferrumequinum –*
647 *Rhinolophus affinis – Aselliscus stoliczkamus*[34], K) *Myotis myotis*[47]. Colors in plots represent different
648 years within study, year 1 (red), year 2 (blue), year 3 (green), year 4 (purple), and year 5 (orange). Black
649 stars show sampling events without detection of coronaviruses. Circles show the mean prevalence and
650 bars the 95% confidence intervals estimated by the Wilson method.

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657 **Box 1: Pathways to pandemic emergence of bat coronaviruses**

658 While the zoonotic potential of an animal virus depends on its ability and opportunity to infect humans,
659 pandemic potential depends on human-to-human transmissibility, quantified by the virus's reproduction
660 number in humans, *R*. The critical value for *R* is 1, the level at which each case replaces itself on average.
661 For subcritical viruses with $R < 1$, transmission chains inevitably die out. For supercritical viruses with $R >$
662 1, epidemics and pandemics are possible[139].

663 Novel viruses with pandemic potential can reach humans by several routes. A virus circulating in bats
664 could have the traits needed for supercritical transmission in humans, by chance or due to evolutionary
665 pressures in the reservoir that fortuitously align with fitness in humans[160]. Such a virus could spill over
666 directly from bats to humans, overcoming ecological barriers of limited spatial overlap and contacts
667 between these species (panel A). Alternatively, such a virus could reach humans via a bridge host that
668 has greater contact with humans, and perhaps also serves to amplify the virus to high levels to increase
669 the probability of initial infection (panel B).

670 Another possibility is that a virus circulating in bats would be subcritical in humans, but has opportunity
671 to evolve to become supercritical within a bridge host that shares some key traits (e.g. homologous
672 receptor proteins) with humans (panel C). A fourth possibility, not depicted here, is that a subcritical
673 virus reaches humans and evolves to become supercritical before its transmission chains die out[160].

674 In any of these scenarios, epidemiologic factors (and simple chance) will determine whether the
675 supercritical virus goes on to cause an epidemic or pandemic. Many such introductions die out,
676 particularly if transmission is highly heterogeneous[43]. Reconstruction of outbreak origins hinges on
677 availability of data and samples from the earliest human cases, and extensive sampling of all involved
678 host species (which often are not known with confidence). Origins and emergence pathways will remain
679 obscure until such data are obtained and analyzed.

680 **Box 2: Spillover of coronaviruses in other species**

681 Coronaviruses have a demonstrated ability for spillover not only involving bats but also transmission
682 among other animal species. For example, HKU2, a coronavirus related to rhinolophid bats, caused an
683 outbreak of fatal disease in domestic pigs in China in 2016 (SADS-CoV; Figure 3)[11]. In 2017, camel
684 (HKU23) and equine (ECoV) coronaviruses were detected in asymptomatic domestic horses in Saudi
685 Arabia and Oman[161]. In 2020, chicken (CdCoV), duck (DdCoV), pigeon (PdCoV), and goose (GdCoV)
686 coronaviruses were observed in live-poultry markets in China, where each of the viruses was found in
687 species of birds other than its primary host[162]. In the 1980s, a fatal outbreak of feline infectious
688 peritonitis in cheetahs (*Acinonyx jubatus*) was caused by a feline coronavirus (FeCoV) that circulates in
689 domestic cats[163]. Within feline CoVs, type II FeCoV emerged from recombination between type I FeCoV
690 and canine-CoV[83,164], highlighting the potential role of co-infection in new hosts in the emergence of
691 new coronaviruses.

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696 **10 References**

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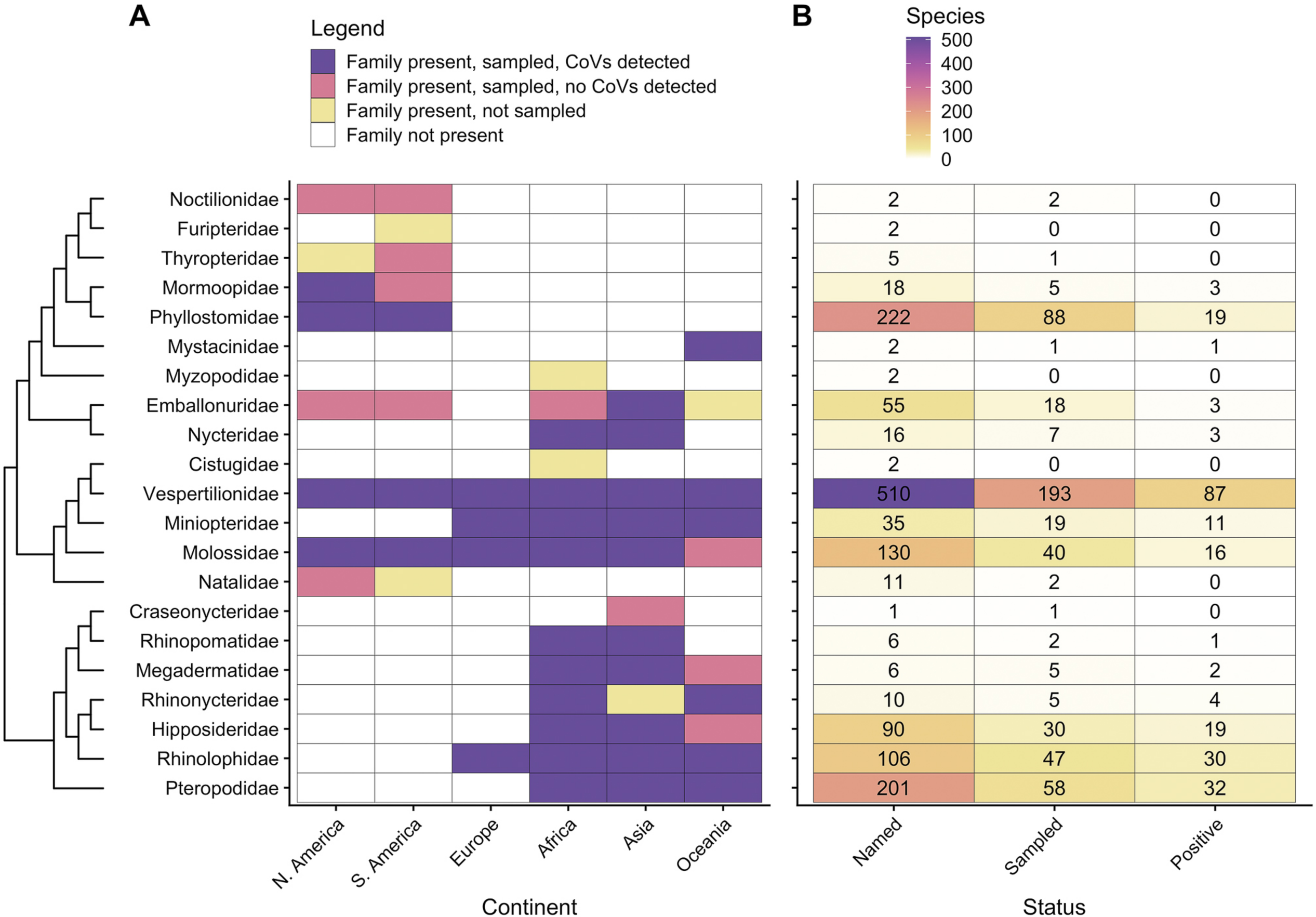
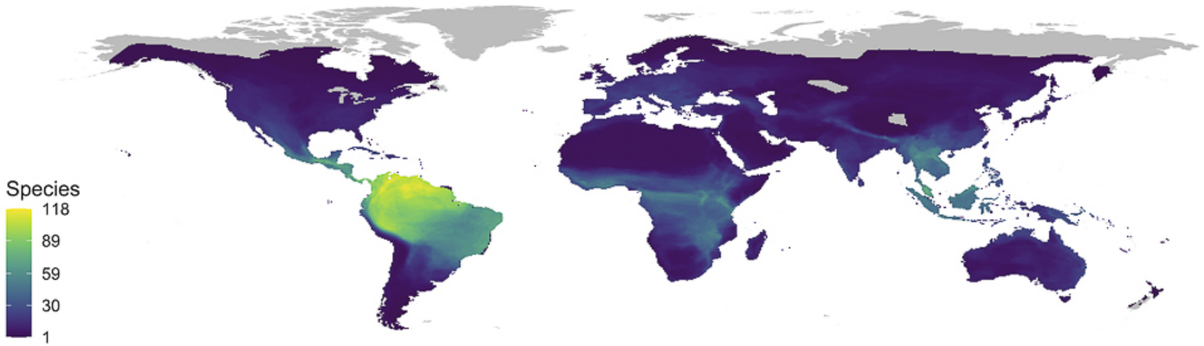
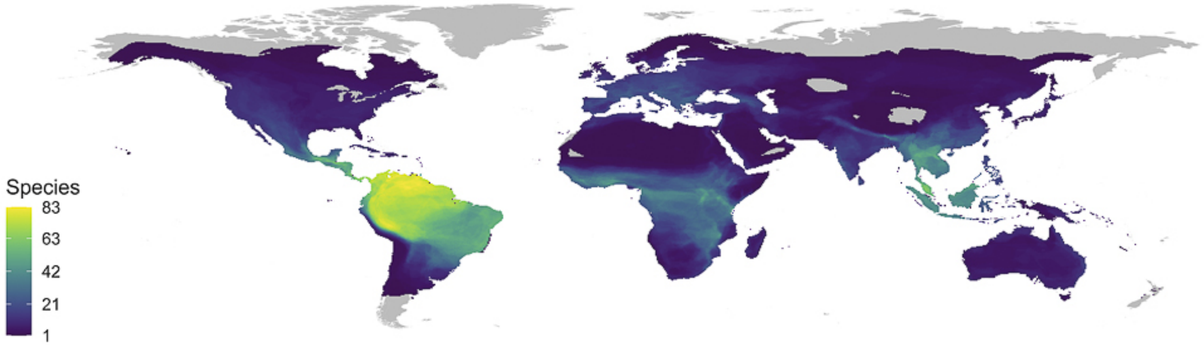
1043


A
Legend
Family present, sampled, CoVs detected
Family present, sampled, no CoVs detected
Family present, not sampled
Family not present
B
Species
500
400
300
200
100
0
Noctilionidae
Furipteridae
Thyropteridae
Mormoopidae
Phyllostomidae
Mystacinidae
Myzopodidae
Emballonuridae
Nycteridae
Cistugidae
Vespertilionidae
Miniopteridae
Molossidae
Natalidae
Craseonycteridae
Rhinopomatidae
Megadermatidae
Rhinonycteridae
Hipposideridae
Rhinolophidae
Pteropodidae
N. America
S. America
Europe
Africa
Asia
Oceania
Continent
Named
Sampled
Positive
Status
2 2 0
2 0 0
5 1 0
18 5 3
222 88 19
2 1 1
2 0 0
55 18 3
16 7 3
2 0 0
510 193 87
35 19 11
130 40 16
11 2 0
1 1 0
6 2 1
6 5 2
10 5 4
90 30 19
106 47 30
201 58 32

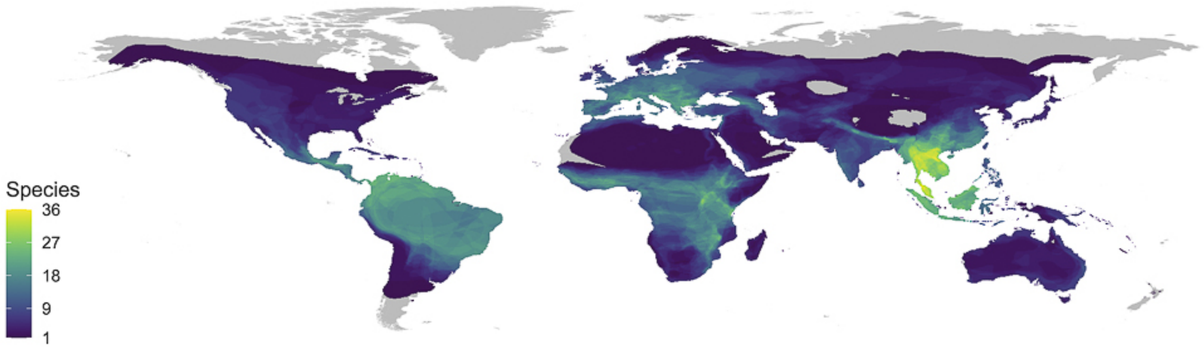
Bat species



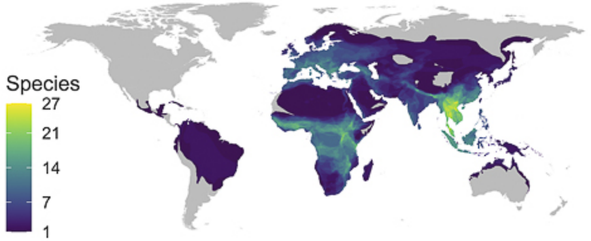
Sampled species



Coronavirus hosts



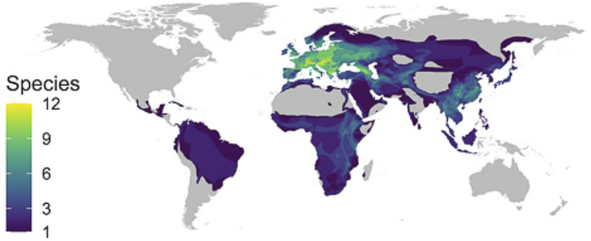
Key coronavirus subgenera hosts



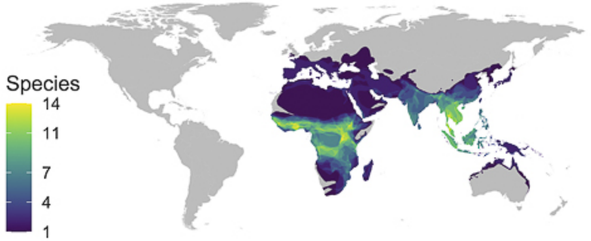
Duvinacovirus hosts

Hibecovirus hosts

Merbecovirus hosts

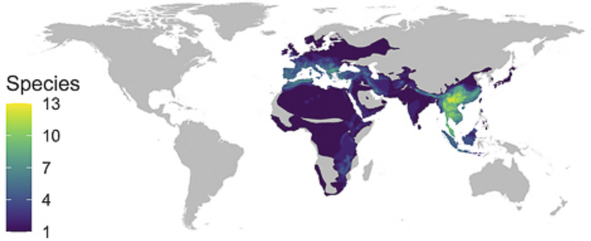


Nobecovirus hosts

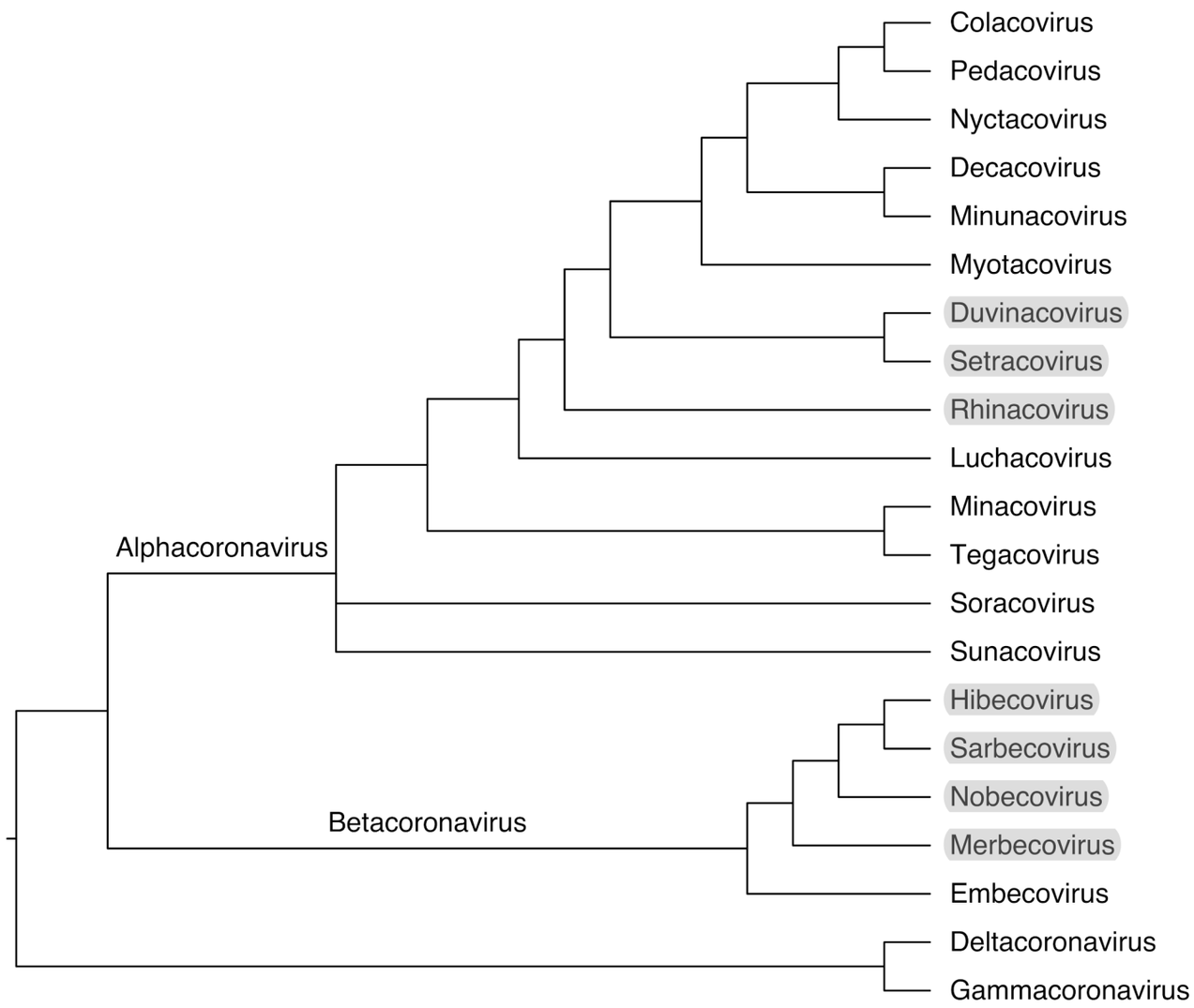


Rhinacovirus hosts

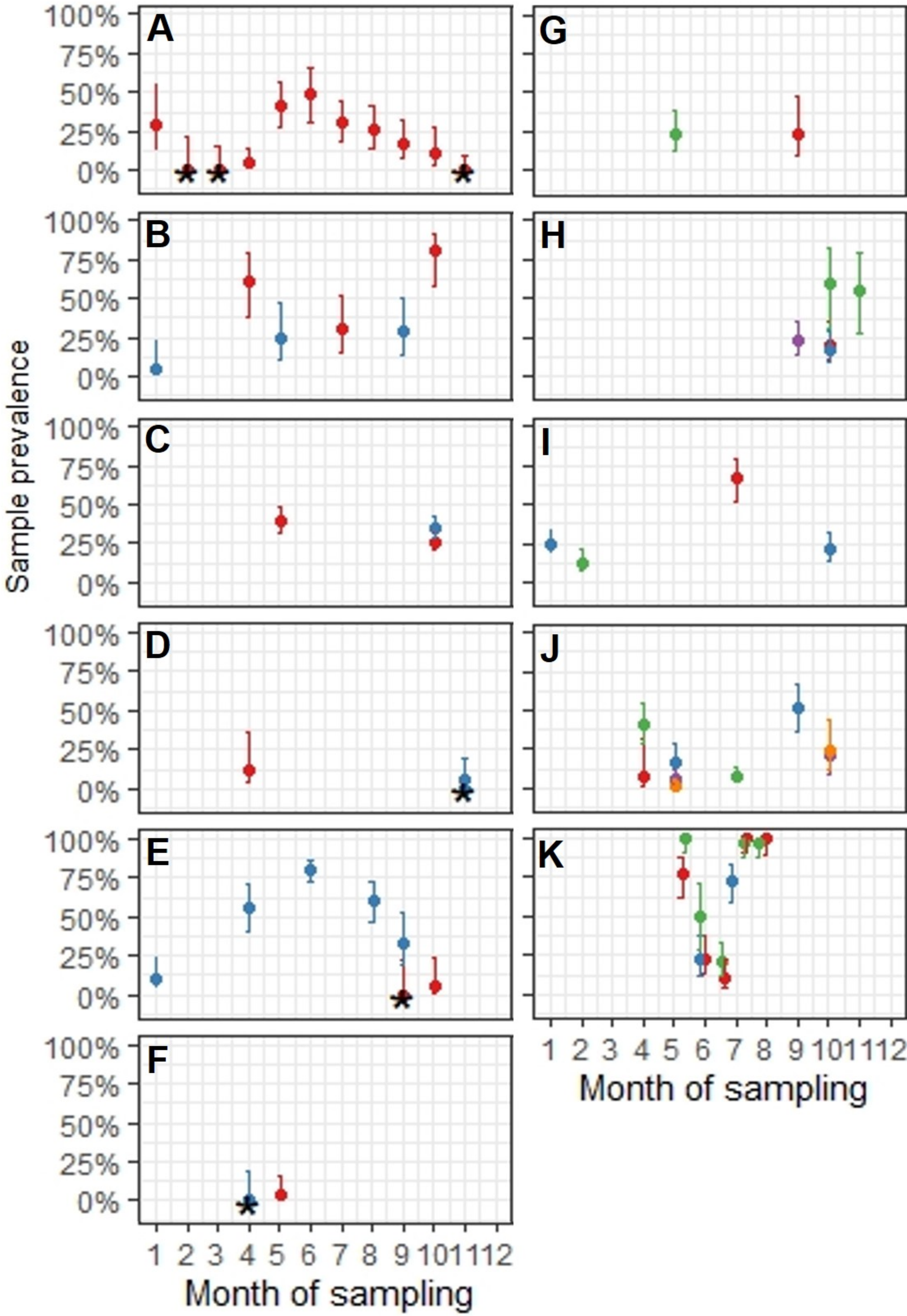
Sarbecovirus hosts

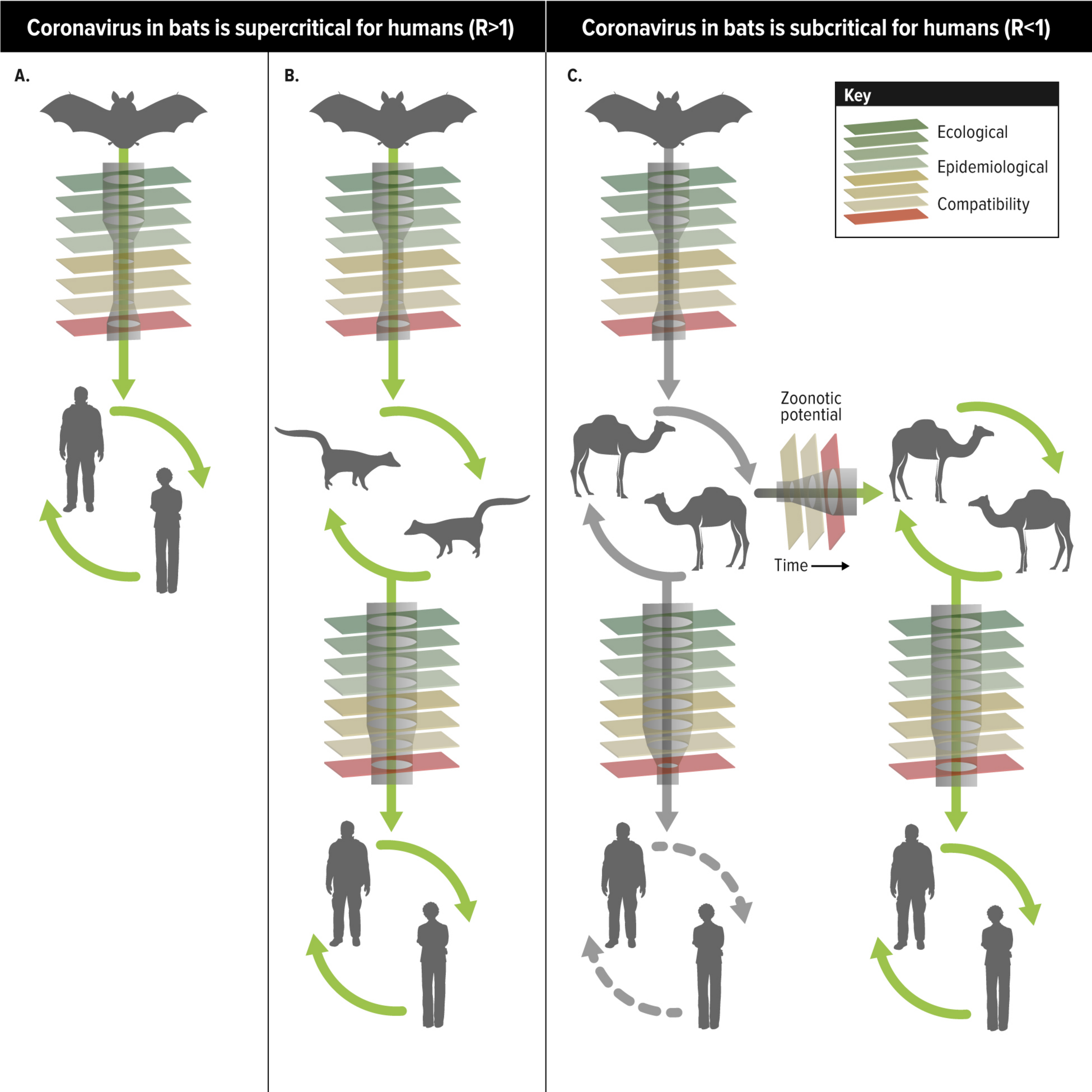


Setracovirus hosts



Genus/ Subgenus	Notable virus species	Host species
Alphacoronavirus		
Colacovirus	Myl-CoV	bats (Vespertilionidae)
Pedacovirus	PEDV	bats (Vespertilionidae), pig
Nyctacovirus		bats (Vespertilionidae)
Decacovirus		bats (Hipposideridae, Rhinolophidae)
Minunacovirus		bats (Miniopteridae)
Myotacovirus		bats (Vespertilionidae)
Duvinacovirus	HCoV-229E	bats (Hipposideridae), dromedary camel, alpaca, human
Setracovirus	HCoV-NL63	bats (Rhinonycteridae), human
Rhinacovirus	SADS-CoV	bats (Rhinolophidae), pig
Luchacovirus		rodents (Muridae, Cricetidae)
Minacovirus		ferret, mink
Tegacovirus	CCoV, FCoV, TGEV	cat, dog, pig
Soracovirus		shrew (*Suncus murinus*)
Sunacovirus		shrew (*Sorex araneus*)
Betacoronavirus		
Hibecovirus		bats (Hipposideridae)
Sarbecovirus	SARS-CoV, SARS-CoV-2	bats (Rhinolophidae), Malayan pangolin, carnivores (Canidae, Felidae, Mustelidae, Viverridae), human
Nobecovirus		bats (Pteropodidae)
Merbecovirus	MERS-CoV	bats (Vespertilionidae), dromedary camel, human
Embecovirus	BCoV, CRCoV, HCoV-OC43, HCoV-HKU1, MCoV	rodents (Muridae, Cricetidae), dog, rabbit, cattle, horse, pig, sable antelope, dromedary camel, giraffe, human
Deltacoronavirus	PorCoV-HKU15	birds, pig
Gammacoronavirus	IBV	birds, cetaceans


A
B
C
D
E
F
G
H
I
J
K
100%
75%
50%
25%
0%
Sample prevalence
1 2 3 4 5 6 7 8 9 10 11 12
Month of sampling


Coronavirus in bats is supercritical for humans (R>1)
Coronavirus in bats is subcritical for humans (R<1)
A.
B.
C.
Key
Ecological
Epidemiological
Compatibility
Zoonotic potential
Time