Recent evolutionary origin and localized diversity hotspots of mammalian coronaviruses

Renan Maestri[a,b,1], Benoît Perez-Lamarque[a,c,1], Anna Zhukova[d], Hélène Morlon[a]

[a]Institut de Biologie de l'École Normale Supérieure (IBENS), École Normale Supérieure, CNRS, INSERM, Université PSL, Paris, France

[b]Departamento de Ecologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

[c]Institut de Systématique, Évolution, Biodiversité (ISYEB), Muséum national d'histoire naturelle, CNRS, Sorbonne Université, EPHE, UA, Paris, France

[d]Institut Pasteur, Université Paris Cité, Bioinformatics and Biostatistics Hub, Paris, France

[1]R.M. and B.P.-L. contributed equally to this work.

*Corresponding authors: Renan Maestri and Benoît Perez-Lamarque

Email: renanmaestri@gmail.com and benoit.perez.lamarque@gmail.com

Author Contributions: R.M. and H.M. conceived the study; R.M, B.P-L., A.Z. and H.M. designed and performed the research; B.P-L. and R.M. analyzed the data; R.M, B.P-L., A.Z. and H.M. wrote the paper.

Competing Interest Statement: The authors declare no competing interest.

Keywords: coronavirus evolution | diversity of coronaviruses | preferential host switches | parasite diversification

This PDF file includes:

Main Text
Figures 1 to 5

Abstract

Several coronaviruses infect humans, with three, including the SARS-CoV2, causing diseases. While coronaviruses are especially prone to induce pandemics, we know little about their evolutionary history, host-to-host transmissions, and biogeography, which impedes the prediction of future transmission scenarios. One of the difficulties lies in dating the origination of the family, a particularly challenging task for RNA viruses in general. Previous cophylogenetic tests of virus-host associations, including in the Coronaviridae family, have suggested a virus-host codiversification history stretching many millions of years. Here, we establish a framework for robustly testing scenarios of ancient origination and codiversification *versus* recent origination and diversification by host switches. Applied to coronaviruses and their mammalian hosts, our results support a scenario of recent origination of coronaviruses in bats and diversification by host switches, with preferential host switches within mammalian orders. Hotspots of coronavirus diversity, concentrated in East Asia and Europe, are consistent with this scenario of relatively recent origination and localized host switches. Spillovers from bats to other species are rare, but have the highest probability to be towards humans than to any other mammal species, implicating humans as the evolutionary intermediate host. The high host-switching rates within orders, as well as between humans, domesticated mammals, and non-flying wild mammals, indicates the potential for rapid additional spreading of coronaviruses across the world. Our results suggest that the evolutionary history of extant mammalian coronaviruses is recent, and that cases of long-term virus–host codiversification have been largely over-estimated.

Introduction

Coronaviruses are RNA-viruses of the family Coronaviridae, comprising positive-sense and single-stranded viruses that have the largest genomes among nidoviruses (1, 2). As several other RNA viruses, they may cause diseases in humans and other animals (3). Depending on the taxonomic arrangement, seven (4–6) or eight (7) species of coronaviruses infect humans, three of which being pathogenic: the SARS-CoV (8, 9), the MERS-CoV (10), and the SARS-CoV2 (11). The latter is at the origin of the recent COVID-19 pandemic that infected more than 620 million people and caused the death of more than six and a half million (12). Coronaviruses' high frequency of recombination (13), broad host range, and high mutation rates (7) make them especially prone to causing yet future diseases. Nevertheless, their evolutionary history and biogeography are very poorly understood. Resolving the evolutionary origins of Coronaviridae, understanding how they diversified, and characterizing their geographic diversity patterns would facilitate attempts to predict future zoonoses (7, 14, 15).

Coronaviruses infect mammals, birds, and fish (2), although they predominate in mammalian species (16–20). A consensus exists on the taxonomic segregation of four genera within Coronaviridae: Orthocoronavirinae, namely Alpha-, Beta-, Gamma- and Deltacoronavirus (2, 21). Alpha- and Betacoronaviruses are found exclusively in mammals, while Delta- and Gammacoronaviruses infect mostly birds but also mammals to a lesser extent (20, 22, 23). Coronaviruses are most numerous and genetically diversified in mammals (2, 23), in particular bats, suggesting a mammalian origin in bats (2, 20, 23, 24), although this remains to be tested.

The timing of origination of the Coronaviridae family is debated, with results that vary by several orders of magnitude. Woo et al (23) found a recent origin, around 10 thousand years ago. This dating was obtained by sequencing the well-conserved RNA-dependent RNA-polymerase (RdRp) genome region of representatives of all four coronavirus genera, and fitting to these sequences a neutral nucleotide-based substitution model with an uncorrelated log-normal relaxed clock (25) calibrated with serial samples. This calibration provided a mean substitution rate estimate of 1.3 x 10-4 substitutions per site per year. Wertheim et al. (26) used this estimate and the same genome region (RdRp), but with a codon-based substitution model accounting for the effect of

selection. Indeed, purifying selection can lead to an underestimation of viral origins when not accounted for (26, 27). They found an ancient origin, around 293 (95% confidence interval, 190 to 489) million years ago (26). More recently, Hayman & Knox (28) obtained similar results, but using the splitting times of hosts as constraints, therefore assuming a priori that coronaviruses codiversified with their hosts.

More generally, dating the phylogenies of RNA virus families is a difficult task (29). While for some of them dated calibration points can be used, based on orthologous copies of endogenous virus elements (EVEs) present in the genomes of related mammalian species with known times of divergence (30), in many others, including in the Coronaviridae, such elements have not been found (31). Despite the difficulty in dating viral families, it has been proposed, from cophylogenetic analyses investigating the congruence of the host and viral phylogenetic trees (32), that vertebrate-associated RNA viruses have codiversified with their hosts over hundreds of millions of years (31, 33). Indeed, RNA virus phylogenies tend to mirror that of their hosts; for example, closely-related coronaviruses infect closely-related mammals (e.g. (28)). However, a major caveat is that such cophylogenetic signals can emerge when viruses diversify by host switches preferentially occurring among closely-related hosts, in the absence of any cospeciation event (34). Event-based cophylogenetic methods can in principle identify cospeciation and host switches events (32, 34), but their behavior in the presence of diversification by preferential host switches is not well understood. Under a perfect codiversification scenario, host and symbiont phylogenies would be identical. Events of host switches, duplications and losses induce mismatches, and cophylogenetic methods aim to identify parsimonious sets of events that allow “reconciling” the two phylogenies (34, 35). However, most of these methods rely entirely on tree topology (and not branching times), such that time-inconsistent host switches between non-contemporary host lineages are allowed during the reconciliation. In the presence of preferential host switches, these methods may thus favor biologically unrealistic reconciliations that involve cospeciation events and ‘back-in-time’ host switches to reconciliations that involve more frequent contemporary host switches. This would have remained unnoticed, unless users of the methods specifically looked at the time consistency of the inferred host switches, which is usually not done.

Here, we establish a framework for testing scenarios of ancient origination and codiversification versus recent origination and diversification by host switches that combines probabilistic cophylogenetic models and biogeographic analyses (Fig 1). We then apply this framework to the Coronaviridae-mammals association. We assemble a dataset of all mammalian hosts of coronaviruses and a complete association matrix between host species and species-like Operational Taxonomic Units (sOTUs) of coronaviruses, as well as geographic repartition of Coronaviridae and their mammalian hosts. We construct a new Coronaviridae tree based on a recent proposition for the use of a well-conserved region of their RNA genome (36, 37). Under the ancient origination scenario (Fig 1A), long-term vertical transmission of Coronaviridae within mammalian lineages could lead to events of mammal-coronavirus cospeciations. Coronaviruses’ diversification would then be modulated by both cospeciations and horizontal host switches from one mammalian lineage to another (26, 31). The most recent common ancestor of coronaviruses could even have infected the most recent common ancestor of mammals and birds (26). Under the recent origination scenario (Fig 1B), codiversification with hosts is virtually impossible, and coronaviruses’ diversification would then be largely dominated by recent host switches. Expectations for the output of reconciliation and biogeographic analyses under these different scenarios, as well as a scenario of random associations, are explicated in Fig 1. We identify the likely origination of coronaviruses in the mammalian tree, quantify the frequency of cospeciation

and host-switching events, and locate these host switches, therefore identifying 'reservoirs' of Coronaviridae and potential transmission routes across mammals.

Results

By screening the 46 sOTUs of Coronaviridae identified by Edgar et al. (36) in public databases, we found 35 that were associated with mammalian hosts. Our trees of these 35 sOTUs support a well-defined split between Alphacoronaviruses and the other genera, regardless of the phylogenetic method used (Fig 2; *SI Appendix*, Fig. S1). Overall, Alphacoronaviruses form a monophyletic clade, Delta- and Gammacoronoviruses form sister clades, with the main uncertainty being on the placement of their ancestor in relation to Beta (i.e. as a sister to a monophyletic Beta-clade (Fig. 2) or within the Beta-clade (*SI Appendix*, Fig. S1).

We found that mammalian hosts of coronaviruses belong to 31 families and 10 orders of mammals, and are widely distributed throughout the mammalian phylogeny (Fig. 2, SI Appendix, Fig. S2). Most mammalian hosts are bats (Chiroptera - 55 species), followed by rodents (Rodentia - 22 species), artiodactyls (Artiodactyla - 15 species), carnivores (Carnivora - 11 species), and primates (Primates - 5 species). Five other orders have at least one representative species: Eulipotyphla (4), Lagomorpha (1), Perissodactyla (1), Pholidota (1), and Sirenia (1). The number of mammalian hosts per coronavirus' sOTU varies across the Coronaviridae tree, ranging from 1 to 22 species, with an average of 4.94 (Fig. 2). Of the 35 sOTUs, 23 are found in at least one bat species and 17, mostly in alphacoronaviruses, are found exclusively in bats (Fig. 2). Eight sOTUs are found in humans, six of which, including the three pathogenetic sOTUs, are betacoronaviruses. Betacoronaviruses infect a larger average number of hosts and a larger diversity of non-bat species than Alphacoronaviruses. Twenty-two coronaviruses occur in more than one species; of those, 11 are found in multiple orders (Fig. 2; *SI Appendix*, Fig. S3) and 11 in multiple species of a single order (*SI Appendix*, Fig. S3). A simple visualization of the mammal-coronavirus interaction network indicates that mammals from the same orders tend to be infected by the same coronavirus' sOTUs, that bats tend to host a specific set of coronaviruses, and the centrality of humans in the network (Fig. 3).

We first tested whether closely-related coronaviruses tend to infect closely-related mammals. A negative answer to this question would suggest that the diversification of Coronaviridae is independent of mammalian history, excluding the scenarios of codiversification or diversification per preferential host switches (Fig. 1). To the contrary, we found a significant phylogenetic signal for the overall association between coronaviruses and mammals (Mantel test: r= 0.38; P= 0.0001) and vice-versa (r= 0.29; P= 0.0001), after accounting for the confounding phylogenetic signal in the number of partners (38). Mantel tests across sub-clades of both phylogenies revealed that this overall phylogenetic signal is linked to phylogenetic signal in the deep nodes of the Coronaviridae and mammal phylogenies rather than at shallow phylogenetic scales (*SI Appendix*, Fig. S4), consistent with the order-level pattern observed in the mammal-coronavirus interaction network (Fig. 3). This pattern could arise from ancient codiversification followed by un-preferential host switches, or from recent host switches preferentially occurring between hosts from the same high-level taxonomic grouping (such as mammalian orders). We also found that closely related coronaviruses tend to infect a similar number of hosts (r= 0.29; P=0.002), while closely related mammals do not tend to host a similar number of distinct coronaviruses (r= 0.04, P=0.1), suggesting that coronaviruses' specificity towards hosts is evolutionarily conserved while hosts' specificity to coronaviruses is not, which may be due to a evolutionarily-labile susceptibility or to the existence of geographic barriers that limit the spread of coronavirus among closely-related susceptible but isolated mammal species.

To further investigate the hypotheses of ancient codiversification versus recent host switches, we used a probabilistic cophylogenetic model, the amalgamated likelihood estimation

178 (ALE - (39)), that reconciliates the host and symbiont phylogenies using events of cospeciations,
179 host switches, duplications, or losses, while accounting for phylogenetic uncertainty in the symbiont
180 phylogenies and undersampling of the host species (35, 39, 40). The main version of ALE we used
181 is an "undated" version that accounts for topology but not branch lengths, as the dated version did
182 not perform well on our data (see Methods). Time-inconsistent host switches are thus allowed
183 during the reconciliation. If the scenario of ancient diversification holds, we expect to find
184 reconciliations requiring more cospeciations and fewer host switches than expected under a
185 scenario of independent evolution (hereafter referred to as 'significant reconciliation'), and few time-
186 inconsistent switches (Fig. 1A). Under the alternative scenario of recent origination and
187 diversification by preferential host switches, we also expect to infer a significant reconciliation, but
188 with many time-inconsistent switches, as the algorithm tends to explain the cophylogenetic signal
189 in the interactions by cospeciation events (Fig. 1B). We indeed found a significant reconciliation
190 between the Coronaviridae and the mammalian trees, confirming the non-independence of their
191 evolution, which we evaluated by randomly shuffling mammal species across the full tree or within
192 biogeographic regions (*SI Appendix*, Fig. S7). ALE reconciliations inferred average numbers of 145
193 cospeciations, 65 losses, 0 duplication, and 92 host switches. Without investigating the time-
194 consistency of the host switches, we would conclude that there are almost 1.5 more diversification
195 events of Coronaviridae that are related to ancient codiversification rather than host switches.
196 However, on average 20% of the inferred host switches are time-inconsistent, including "back-in-
197 time" host switches of >50 Myr (*SI Appendix*, Fig. S8). Similar results were observed when
198 accounting for the uncertainty in the branching time estimates (*SI Appendix*, Fig. S8), which
199 suggests instead that extent Coronaviridae originated recently and diversified by frequent
200 preferential host switches. 64% of the reconciliations found an origination of coronaviruses within
201 bats, in particular within the Pteropodidae family (Fig. 4A-C). By comparison, only 19% found an
202 origination in rodents, 6% in artiodactyls, and 2% in carnivores. In addition, we no longer found an
203 origination in bats when randomly shuffling the dataset (*SI Appendix*, Fig. S5,S6), suggesting that
204 this result is not artifactual. We checked the interpretation of our results by simulating the two
205 scenarios of (i) ancient origination in the ancestors of bats followed by codiversification and (ii)
206 recent origination in an extant bat species and a subsequent diversification by preferential host
207 switches. On the first set of simulations, ALE correctly inferred an origination in bats and very few
208 time-inconsistent switches (2% +/- 2%), which seems to be the basal expected proportion of time-
209 inconsistent switches under a scenario of codiversification, *SI Appendix*, Fig. S12). On the second
210 set, ALE correctly inferred an origination in bats, although with lower confidence, and a large
211 fraction (~20%) of time-inconsistent host switches, similar to what we observed for Coronaviridae.
212 These results therefore indicate a scenario of recent origination of coronaviruses in bats followed
213 by diversification by preferential host switches.
214
215 To investigate this scenario in more detail, we gradually applied a tree transformation to
216 the mammalian phylogeny, which excludes the possibility of an ancient origination happening
217 earlier than a given time. We found that we had to impose a very recent time of origination (younger
218 than 5 Myr) to obtain few time-inconsistent switches (*SI Appendix*, Table S1). We thus carried out
219 our follow-up analyses with a mammals' tree transformation (star phylogeny) that assumes an
220 origination in an extant mammalian lineage, such that coronavirus diversification is explained
221 entirely by host switches between extant mammalian species. Simulations validated this approach
222 in terms of properly inferring originations and identifying preferential host switches (*SI Appendix*,
223 Fig. S13). Applied to the data, the approach inferred a high probability of origination in bats (56%,
224 Fig, 4B-C, *SI Appendix*, Fig. S6), far more likely than in other mammalian orders (artiodactyls: 18%,
225 rodents: 7%, carnivores: 5%). Results were consistent when subsampling only up to 10 mammalian
226 species per order, suggesting than the likely origination in bats is not driven by the high number of
227 bats species in the dataset (*SI Appendix*, Supplementary Information Text). Simulations also
228 confirmed that our results did not spuriously arise because of higher coronaviruses diversification
229 in bats compared to other mammalian orders (Fig. S14). Following the origination in bats, the
230 approach inferred a scenario of diversification by preferential host switches: 68% of the inferred
231 host switches happened within mammal orders (Fig 4D, *SI Appendix*, Fig. S10), whereas we would

expect on average only 28% of within-order host switches if happening at random. We also inferred more-than-expected host switches between closely related mammal orders (e.g. between Artiodactyla and Perissodactyla) and between the order containing humans (Primates) and those of their domesticated animals, such as Artiodactyla and Carnivora (*SI Appendix*, Fig. S10, Table S2). In contrast, host switches were five times less numerous than expected by chance between bats and other orders (10.7%, against 50.2% on average if host switches were randomly distributed, Fig. 4D), in particular Artiodactyla and Rodentia (*SI Appendix*, Fig. S11, Table S2). When occurring, host switches from bats often occurred toward humans (1.9 host switches per reconciliation on average) or toward urban-living and/or domesticated animals, such as rats, camels, or pigs (>1 host switch on average; *SI Appendix*, Table S3). Host switches to humans occurred mostly from domesticated mammals (camels, pigs, dogs), the house shrew and the house mouse, then followed by Asian palm civets, and lastly by bats and other rodents (*SI Appendix*, Table S4). Results were consistent when subsampling only up to 10 mammalian species per order or when subsampling the dataset to have an equal sampling effort per host species, suggesting that our results are not artifactually explained by the enhanced monitoring of coronaviruses in humans or domesticated animals (*SI Appendix*, Supplementary Information Text). Finally, we found that some sOTUs, in particular from Betacoronaviruses (e.g, u24667 and u175, both with humans among their hosts), have experienced frequent host switches, whereas others have not (e.g. u165, which is restricted to pigs). In particular, u944 (SARS-Cov-2) has experienced an intermediate number of host switches compared to other coronaviruses (*SI Appendix*, Fig. S9).

We found qualitatively similar results when applying ALE on different sub-parts of the palmprint region, suggesting that the potential occurrence of recombination does not bias our conclusions (*SI Appendix*, Table S5). The percentage of originations inferred to occur in bats decreased in the analyses on the first sub-part, probably because using such a short fragment (75 aa-long) does not allow robust reconciliations. We also obtained consistent results using a reconciliation method based on maximum parsimony (eMPRess) instead of maximum likelihood (ALE). Whatever the costs that we set for the different reconciliation events, eMPRess estimated significant reconciliations (p-values<0.01). For instance, when favoring host switches, we inferred a recent origination in bats in 54% of the reconciliations and observed on average 32 cospeciations (s.d. +/-3), 2 losses (s.d. +/-1), 0.1 duplication (s.d. +/-0.3), and 140 host switches (s.d. +/-3) including several “back-in-time” host switches of >30 Myr. eMPRess therefore also supports a scenario of recent origination in bats and diversification by preferential host switches (Fig. 1B). Without investigating the time-consistency of the host switches, we would have wrongly concluded that almost one fourth of the diversification of Coronaviridae is related to ancient cospeciation events.

An additional piece of evidence for a recent origination scenario comes from the geographical distribution of coronaviruses, with a hotspot of diversity in Eurasia that has not colonized the whole world (Fig. 5A, Fig. 1B). The coronavirus’ hotspot is more strongly influenced by the diversity of alphacoronaviruses than of betacoronaviruses (*SI Appendix*, Fig. S15). The higher host switches rates and broader host range of betacoronaviruses is reflected in a more widespread geographic distribution, with less pronounced hotspots when compared to alphacoronaviruses (*SI Appendix*, Fig. S15). Mammalian hosts of coronaviruses have a hotspot of species diversity concentrated in East Asia (Fig. 5C). The richness of coronaviruses presents a similar pattern, but with two comparable hotspots of species diversity in East Asia and Southern Europe (Fig. 5A), suggesting that the European hotspot is composed by fewer host species, together carrying as diverse a set of coronaviruses as the Asian hotspot. Other regions with a relatively high richness of coronaviruses and their hosts include parts of the African continent. The Americas and Australia have relatively low richness of coronaviruses and their hosts. Phylogenetic

diversity of both hosts and coronaviruses (Fig. 5B, D) depict a similar pattern but with phylogenetic diversity more evenly distributed across most world regions, including the Americas.

Discussion

Together, our results suggest that the common ancestor of extant mammalian coronaviruses originated recently in a bat species, and that coronaviruses diversification occurred via preferential host switches rather than through codiversification with mammals. Although we cannot unequivocally reject that ancestors of present-day coronaviruses were not present several million years ago, we demonstrate that Coronaviridae is a highly dynamic clade in which diversification operates through host switches at a much faster pace than that of their hosts. sOTUs are rapidly replaced by newly-generated ones, with little role for codiversification with the hosts. The high diversity and endemicity of coronaviruses among bats have led others to anticipate that bats might be implicated in the origin of coronaviruses (2, 20, 23, 24), although definitive proof was lacking. We provided evidence for that hypothesis using a probabilistic cophylogenetic model and accounting for the entire known diversity of coronaviruses across mammals. Independent evidence for coronavirus recent host switches among different species exists in the literature (41, 42). The envisioned scenario suggests a timing of origination for extant Coronaviridae that is much more recent than the hundreds of millions of years ago suggested by (26). This is not surprising given the difficulties in estimating divergence times and inferring branch lengths for viral phylogenies (26, 27, 29), and provided that the dating of Wertheim et al. (26) relied on a substitution rate estimated from data with limited temporal signal (~50 serially sampled contemporary sequences of a short gene fragment, (23)).

Our results contradict previous suggestions that codiversification with vertebrate hosts played an important role in Coronaviridae diversification (26, 28, 31). They also suggest that previously reported cases of long-term codiversification in vertebrate RNA viruses have been largely over-estimated, as many of them may instead be cases of diversification by host switches occurring preferentially among closely-related hosts. Indeed, these two scenarios both generate cophylogenetic signal in host-symbionts associations, such that cophylogenetic signal alone is not evidence for long-term codiversification (34). In addition, under a scenario of recent origination and preferential host switches, event-based cophylogenetic methods tend to artifactually favor biologically unrealistic scenarios with codiversification and back-in-time host switches, as we have shown here. As the time-consistency of host switches is typically not investigated, this has remained unnoticed, and evidence for codiversification has been taken for real. Ideally, cophylogenetic reconciliation methods would not allow such time-inconsistent host switches. However, imposing time constraints in methods based on parsimony is NP-hard (43), and the 'dated' version of ALE is not well adapted when recent host switch events dominate evolutionary history. We have found two ways to get around the problem, by interpreting time-inconsistent host switches as evidence for recent preferential host switches, and by gradually transforming the host tree to avoid large back-in-time switches, however future efforts should focus on developing time-consistent cophylogenetic methods. This would allow more robust and precise inferences of host-virus (and more generally host-symbiont) evolutionary history.

During their evolution, coronavirus' host switches occurred more frequently within than between mammalian orders. This suggests that mammalian characteristics shared between relatives (e.g., genetic, behavioral, ecological), and the frequency of encounters among hosts play important roles in determining coronavirus' host switches. Additionally, between-order host switches occurred more frequently among non-flying mammals and among orders containing humans and urban and domesticated mammals, suggesting that contact frequency alone is likely a key characteristic in host switches. Accordingly, amongst the most-likely host switches towards humans were those coming from mammals suspected to be involved in the transfer of specific coronavirus sOTUs likely through contact, for instance, camels in the case of MERS-CoV (41),

Asian palm civets with SARS-CoV (16, 17) and the house mouse with SARS-CoV-2 (42). Importantly, we found that host switches from bats to other mammalian species were rare during the evolutionary history of Coronaviridae, even though coronaviruses originated and are more diverse within bats. These pieces of evidences suggest that bats are a closed reservoir of the Coronaviridae diversity, as also suggested by their relative isolation in the mammal-coronaviruses interaction network (Fig. 3).

Spillovers from bats to non-bat species, when they occurred, were found more likely to be towards humans than to any other mammalian species, suggesting humans may have acted as evolutionary intermediate hosts amongst mammals, in line with their centrality in the mammal-coronaviruses interaction network (Fig. 3). From an ecological perspective, the large abundance and widespread geographic distribution of humans, together with our habits of forcing contact with other species, including bats, make it unsurprising that humans, among all mammal species, have acted as intermediate hosts of ancestral forms of coronaviruses. Interestingly, for some individual species of coronaviruses, such as the SARS-CoV2 and other SARS-like coronaviruses, the dominant hypothesized scenario is that precursor forms spread from a bat to another intermediate mammalian host before infecting humans (20, 44). Our molecular marker lacks the intra-OTU resolution necessary to make species-level predictions, but our results suggest that more ancient coronaviruses host switches may have occurred in the other direction: from bats to humans to non-bat mammals. The large spillover of SARS-CoV2 from humans to wild mammalian lineages has now been well documented (76) and tend to confirm our results of humans as the intermediate host. Many human activities lend credit to the human-as-evolutionarily-intermediate-host-hypothesis, including human excursions to bat caves (45), hunting (46), and habitat destruction and modification (47), all of which increase the contact between bats and humans and their domesticated animals (47). Conservation of bats' natural habitats, away from human contact, could help avoiding further spreads of coronaviruses among humans.

Insights of past and future host switches are gained from coronavirus geographic distribution. Coronaviruses are found worldwide and their hotspots of diversity are concentrated in East Asia and Southern Europe, where they likely originated. Previous assessments of the diversity of bat hosts of betacoronaviruses suggested similar hotspots but with a distribution of coronaviruses more concentrated in the hotspots (7, 14, 15) than the more pervasive pattern we found using all mammalian hosts. Moreover, the distribution of coronaviruses is less concentrated in the hotspots when phylogenetic metrics of diversity are included, suggesting that species richness alone is masking the global evolutionary potential of these viruses (48). Coronaviruses' likely recent origination in bats, high within-order transmission rates, and their capacity to switch between mammal orders in some cases suggest the potential for future fast spreading and increase in the number of species across most world regions. Among alphacoronaviruses, the spread is more likely to remain concentrated within bats, while betacoronaviruses have a higher potential for among-orders spreading and infection of new mammalian hosts. The betacoronaviruses lineages already detected in humans have especially high host generalism and transmission rates, indicating that these lineages should be particularly monitored to avoid future pandemics.

Finally, a few important limitations of our analyses deserve to be mentioned. First, we used a short marker gene to reconstruct the Coronaviridae phylogeny. However, the RdRp palmprint marker we used is very conserved and ideal for viral species tree construction (36, 37). Moreover, our tree has an identical genus-level topology compared with trees constructed using other genome regions, such as the nucleocapsid portion of the coronavirus genome (23), and very close topologies were also observed using other genome regions such as the spike, envelope, and membrane regions (23), as well as the entire RdRp region (2, 6, 23): it differs only on the placement of Gamma and Deltacoronavirus in relation to the others, but it is consistent in the monophyly of both Alpha and Beta and recovers the sister-clade relationship between Gamma and Delta. Second, the record of associations between coronaviruses and mammals is necessarily incomplete, as not all mammal species have been screened for coronaviruses, and likely biased

towards bats and mammals that are in contacts with humans. However, our sub-sampling analyses suggest that our main results (recent origination in bats and frequent transfer from human-associated mammals to humans) are not due to sampling biases. Third, recombination is an important mechanism of viral evolution (49), and approaches more adequately designed to investigate the role of recombination are needed. The fact that different subparts of the palmprint region lead to similar results indicates that recombination acting on the palmprint region is unlikely to bias our conclusions. However, looking at other genomic regions would allow gaining a more complete understanding of the role of recombination in coronavirus evolution. Lastly, because the palmprint region is a conserved region, we could not reconstruct the recent evolutionary history of coronaviruses (i.e. the within sOTU transmission dynamic): combining the palmprint region with a fast-evolving region(s) would enable more precise estimates of the recent routes of coronaviruses' transmission, including that of SARS-CoV-2. We carried a series of sensitivity analyses all along the manuscript to assess robustness to potential biases or issues, summarized in Table S7.

Understanding the evolutionary origins and diversification of viruses is crucial to any attempt of predicting new transmission routes, yet the relative frequencies of virus–host cospeciation versus cross-species transmission in the evolution of vertebrate RNA viruses remains uncertain (31). We found that coronaviruses originated in bats where they are more diverse nowadays, and later diversified in other mammal orders through preferential host switches. Spillovers from bats were rare but likely human-induced, suggesting humans are the intermediate evolutionary bridge that facilitated the spread of coronaviruses across mammals. Host switches between primates and artiodactyls, perissodactyls, and carnivorans happen at high rates and we can thus expect a spread of coronaviruses amongst new mammalian hosts and outside of their current diversity hotspots in East Asia and Europe, as well as future coronaviruses-related pandemics. Our results suggest reducing human-bat contact, for example by conserving bat habitats, as a mitigation strategy. They also suggest that cases of long-term virus–host codiversification, reported on the basis of cophylogenetic tests, have been largely over-estimated.

Materials and Methods

Operational Taxonomic Units for Coronaviridae

We used the 46 described species-like Operational Taxonomic Units (sOTUs) for Coronaviridae delimited using 'palmprint' sequences by (36, 37). The palmprint is a conserved amino acid (aa) sub-sequence (150 aa in Coronaviridae) of central importance in the viral RdRp (36), selected for its homology across the large majority of sequences, allowing estimation of sequence divergence and phylogenetic trees (37). sOTUs were identified by Edgar et al. (36) after clustering palmprint sequences at 90% amino acid identity; and released through the Serratus project. Their approach is equivalent to the species delimitation proposed by the International Committee on the Taxonomy of Viruses for Coronaviridae ((2); *SI Appendix*, Supplementary Information Text), which suggests 90% similarity of amino acid sequences for conserved domains (2, 37), and are, therefore, ideal for species tree construction. Despite its relatively short length, trees constructed with this region are topologically equivalent to Coronaviridae trees based on other genes (see Discussion). Under the 'palmprint framework' a centroid definition of species is applied to characterize a new OTU when a threshold of 90% amino acid identity is surpassed, serving as a useful taxonomic barcode (37). We downloaded the palmprint amino acid sequences of Coronaviridae sOTUs from the Serratus project (https://serratus.io/; (36)) on April 13 of 2022.

Mammalian hosts of Coronaviridae

All 46 sOTUs of Coronaviridae with a full palmprint and associated data in the NCBI database were screened for the identification of its hosts. From those, 35 sOTUs were associated with mammalian

hosts and were kept for downstream analyses. Serratus' associated metadata was used to identify GenBank accession codes linked to each sOTU. The complete set of 90,540 associated GenBank accession codes was screened to obtain the host information for each sOTU (on NCBI, Features>source>/host=). All the host species with a full Linnean name were kept as such. Accession codes with hosts leading to a generic level information were further inspected to identify the associated publication and determine the complete species name. Dubious cases or accession codes without publications had their hosts disregarded. Common names or high-level host information (e.g., host="bats") were generally eliminated except in a few cases where a domesticated species was found to be the host (i.e., host="dog","canine" were *Canis lupus*; host="cat","feline" were *Felis catus*; host="pig","piglet","newborn piglet","sucking piglet","porcine","swine" were *Sus scrofa*). A final dataset of 116 mammalian hosts associated with the 35 sOTUs was assembled and used in downstream analyses. A matrix with the association between Coronaviridae sOTUs and mammalian species is available in *SI Appendix*, Dataset S1.

Coronaviridae phylogenetic trees

We constructed a Coronaviridae tree using the palmprint amino acid sequence information of the 35 sOTUs. We aligned the amino acid sequences with MAFFT (50) and trimmed them with trimAl (51). The final alignment contained 150 amino acid positions. We used two main phylogenetic software, BEAST2 (52), which performs rooting and time calibration and PhyloBayes (53), which generates outputs adapted to the cophylogenetic algorithm we used. We visualized phylogenetic trees using R (54).

In order to run BEAST2, we generated an input file using BEAUti with 35 sOTU sequences and the following parameters: a WAG model with 4 classes of rates and invariant sites, a birth-death prior, and a relaxed log-normal clock. BEAST2 sampled a posterior distribution of ultrametric trees using Markov chain Monte Carlo (MCMC) with 4 independent chains each composed of 100,000,000 steps sampled every 10,000 generations. We checked the convergence of the 4 chains using Tracer (55). We used LogCombiner to merge the results setting a 25% burn-in and TreeAnnotator to obtain a Maximum Clade Credibility (MCC) tree with median branch lengths.

To further assess the robustness of the BEAST2 tree rooting, we estimated the root position on a 46-sOTU maximum likelihood tree (from the Serratus project - (36)) assuming a strict molecular clock and an ultrametric tree. We used an ultrametric setting as temporary information from the tip dates (ranging between 1999 and 2022, a neglectable difference with respect to the root age of dozens of thousands or even millions of years) was not sufficient to infer the mutation rate (we assessed the temporal signal with TempEst, (56)). We performed rooting and time-scaling with LSD2 (v2.3, (57)), assuming a tree of unknown scale (e.g. fixing all the tips dates to 1 and the root date to 0) with outlier removal and root search on all branches. LSD2 detected no outliers and positioned the root on the same branch as in the BEAST2 MCC tree (between alpha and betacoronaviruses).

The tree we constructed with PhyloBayes was specifically designed to the application of cophylogenetic methods, which cannot handle the presence of the same symbiont species in multiple host species. Following (35), we multiplicated the sOTU sequences in cases when a sOTU was present in several mammal species, such that each mammal-coronavirus association is represented by one single sequence (total of 173 sequences). We reconstructed the phylogenetic tree of the 173 sequences with PhyloBayes, run using an LG model, 4 classes of rates, and a chain composed of 4,000 steps with a 25% burn-in.

Mammalian phylogenetic tree

We obtained a phylogenetic hypothesis for mammals from the consensus DNA-only tree of (58), one of the most complete and updated phylogenies for mammals. We downloaded the node-dated tree for 4,098 mammals, constructed based on a 31-gene supermatrix, from the VertLife website

489 (http://vertlife.org/data/mammals/). We used a pruned version of the tree with the 116 mammalian
490 hosts of Coronaviridae in all analyses in this paper. We kept for each node the 95% credible interval
491 of its age estimate.
492

493 **Phylogenetic signal in the association between coronaviruses and mammals**

494 To assess whether closely related coronaviruses interact with similar mammals, and vice-versa,
495 i.e. presence of phylogenetic signal in the association, we used Mantel tests following (38). Mantel
496 tests were constructed by taking the Pearson correlation between phylogenetic distances and
497 ecological distances. Phylogenetic distances of coronaviruses were computed on the BEAST2
498 MCC phylogeny. Ecological distances were calculated based on the interaction network matrix
499 containing the association between coronavirus' sOTUs and mammals, accounting for the
500 evolutionary relationships among interaction partners using UniFrac distances (59). Firstly, we
501 conducted Mantel tests permuting the identity of species but keeping the number of partners per
502 species constant; this allows for assessing the effect of species identity while controlling for the
503 confounding effect of the number of partners. Then, we evaluated the phylogenetic signal in the
504 number of partners alone. Lastly, we calculated clade-specific Mantel tests for sub-networks
505 containing at least 10 species (38) to evaluate whether phylogenetic signal was stronger for specific
506 subclades of mammals or coronaviruses. Ten thousand permutations were used in each analysis
507 to assess significance. Analyses were conducted using the phylosignal_network and
508 phylosignal_sub_network functions in the R package RPANDA (60).
509

510 **Coronaviridae origination and host switches**

511 We used the amalgamated likelihood estimation (ALE - (39)) to reconciliate the mammal and
512 coronaviruses evolutionary history using events of cospeciations, host switches, duplications, and
513 losses. Originally designed in the context of gene tree – species tree reconciliations (39), ALE has
514 also been particularly useful in the context of host-symbiont cophylogenetic analyses as it considers
515 both phylogenetic uncertainty of the symbiont evolutionary history and undersampling of host
516 species (35, 61, 62). ALE indeed assumes that host switches may imply an unsampled or extinct
517 intermediate host lineage (40). ALE therefore intrinsically accounts for the incompleteness of our
518 dataset, *i.e.* the fact that we only observe a subsample of mammalian species for which
519 coronaviruses have been detected among all the infected species. We ran ALE with the posterior
520 distribution of phylogenetic trees of coronaviruses generated with PhyloBayes to estimate the
521 maximum likelihood rates of host switches, duplications, and losses of the coronaviruses. We first
522 tried running the "dated" version of ALE, which accounts for the order of branching events in the
523 host phylogeny, therefore only allowing for time-consistent host switches (i.e. host switches that
524 happen between two contemporary host lineages). However, this led to unrealistic parameter
525 estimates (such as very high loss rates) and ALE was not able to output possible reconciliations,
526 suggesting that the mammalian and Coronaviridae trees are too incongruent to be reconciliated
527 with only time-consistent host switches, *i.e.* that the scenario of codiversification is likely unrealistic.
528 We therefore used the "undated" version of ALE that only exploits the topology of both the host and
529 the symbiont tree and thus does not constrain the host switches to be time-consistent. ALE
530 generated a total of 5,000 reconciliations, from which we extracted the mean number of
531 cospeciations, host switches, duplications, and losses. We also reported the likely origination of
532 coronaviruses in mammals (i.e. the branch in the mammal phylogeny that was first infected by
533 coronaviruses) by computing, for each branch of the mammalian tree, the frequency of
534 reconciliations (among the 5,000) that supported an origination in that branch. If a reconciliation
535 requires more cospeciation events and fewer host switch events, than expected under a null
536 scenario of independent evolution, this indicates that the evolution of the symbiont was not
537 independent of that of the host, and in this case, we talk about a "significant reconciliation" (63).
538 We evaluated the significance of the reconciliation by comparing the estimated number of

539 cospeciation and host switch events to null expectations obtained with ALE by shuffling the
540 mammal host species across the mammal tree, both randomly or within major biogeographic
541 regions according to the proposal of regions by (64) for mammals (six biogeographic regions: North
542 American, South American, African, Eurasian, Oriental, and Australian). We considered a
543 reconciliation to be significant if the observed number of cospeciations was higher than 95% of the
544 null expectations and if the number of host switches was lower than 95% of the null expectations
545 (35). The likeliness of a host switch between two mammal lineages is measured as the frequency
546 of the reconciliations in which it occurs. Finally, we reported the ratio of time-inconsistent host
547 switches by focusing on "back-in-time" switches, from a donor mammal lineage to an older receiver
548 mammal lineage that never coexisted. We identified time-inconsistent host switches directly on the
549 consensus mammalian phylogeny, or considering the 95% credible interval around each age node
550 estimate to avoid counting time-inconsistent host switches that may arise from incorrect estimates
551 of divergence times.
552
553 Because ALE estimated a large proportion of time-inconsistent host switches (see
554 Results), we first tested the scenario of a more recent origination by collapsing all mammalian
555 nodes anterior to X Myr into a polytomy at the root of the phylogeny (with X varying from 55 Myr to
556 5 Myr), such that the coronavirus origination and host switches inferred by ALE could not involve
557 mammal lineages older than X Myr. Second, we investigated the scenario of diversification by pure
558 preferential host switches of the coronaviruses among extant mammals. To do so, we ran ALE on
559 a star mammalian phylogenetic tree. In this context, ALE could no longer infer cospeciations, and
560 only fit events of host switches, duplications, or losses. When inferring a likely host switch between
561 two specific mammalian lineages on a star phylogeny, there are often as many reconciliations
562 suggesting one directionality of the host switch (i.e. from one of the lineages to the other) as the
563 other. We then only kept host switches present in at least 10% of the reconciliations and looked at
564 the ratio between the number of host switches that were estimated within versus between mammal
565 orders. We compared this ratio to a null expectation obtained by randomly shuffling the host
566 mammal species.
567
568 Recombination is frequent in viruses and the palmprint region may be recombined, such
569 that different fragments of the palmprint region may have different evolutionary histories, potentially
570 biasing our inference. To test whether the results we obtained on the whole 150-amino acid
571 palmprint region were not impacted by recombination, we replicated the ALE analyses on two sub-
572 regions: the first part (positions 1-75) and the last part (positions 76-150).
573
574 Finally, we repeated our cophylogenetic analyses using eMPRess (43), another event-
575 based cophylogenetic approach that reconciliates host-symbiont evolutionary histories using
576 maximum parsimony. eMPRess is a recent improved version of the popular Jane approach (32); it
577 differs from Jane especially by not only relying on a heuristic and therefore guarantying that the
578 solution truly corresponds to the maximum parsimony reconciliation(s) (43). However, contrary to
579 Jane, eMPRess does not offer the possibility to constrain host switches to occur only among
580 lineages from pre-specified time periods. eMPRess requires specifying cost values for the events
581 of host switches (t), duplications (d), and losses (l). We tested two sets of cost values: (1) cost
582 values that disadvantage host switches (d=6, t=6, l=1) and (2) uniform cost values that favor host
583 switches (d=1, t=1, l=1). As with ALE, we evaluated the significance of the reconciliations using
584 permutations. We ran eMPRess analyses on a set of 50 trees randomly sampled from the posterior
585 distribution of PhyloBayes.
586

587 **Sampling biases**

588 More effort has been put on the characterization of coronaviruses associated with humans,
589 domesticated animals, and bats (bats represent 47% of the mammalian species in our dataset, in
590 part because many bat species have been intensively screened for viruses). This can lead to two

main sampling biases that could potentially impact our conclusions: (i) unequal sampling effort across mammalian species that have been screened, and (ii) unequal screening of mammalian species across the mammal tree of life. To assert whether such sampling biases could generate spurious results we designed two subsampling strategies and re-run the PhyloBayes and ALE analyses on each subsampled dataset (*SI Appendix*, Supplementary Information Text).

Simulation analyses

By running the undated version of ALE either on the mammal phylogeny or a star phylogeny, we proposed a framework to evaluate whether the cophylogenetic pattern is due to a history of ancient codiversification (i.e. a mix of cospeciations, host switches, duplications, and losses; Fig. 1A) or to a scenario where the coronaviruses diversify more recently by preferential host switches ((34); Fig. 1B). To validate the interpretation of our ALE results, we performed simulations under the two alternative scenarios of codiversification and diversification by preferential host switches. For the scenario of codiversification, we assumed that coronaviruses originated in the ancestors of bats and that they subsequently codiversified with the mammals by experiencing events of cospeciations, host switches, duplications, and losses. We used the function *sim_microbiota* in the R-package HOME to obtain the corresponding coronavirus sequences and coronavirus-mammals associations (65) (*SI Appendix*, Supplementary Information Text). For the scenario of coronaviruses diversification by preferential host switches, we used a birth-death model (pbtree function in the R-package phytools) to simulate a phylogenetic tree of the coronaviruses: in our model, each coronavirus lineage is associated with a single host species, a birth event corresponds to a host switch (at rate 50), while a death event corresponds to a loss of a coronavirus in a host lineage (at rate 5). We started the diversification by assuming a single coronavirus infection in *Eidolon helvum* (a bat host of external lineages within Betacoroviruses, u25738 and u27845). Then, following de (66) and (67), we modeled preferential host switches by assuming that for a host switch from a given donor mammal species, each potential receiver species has a probability proportional to exp(-0.035**d*) where *d* is the phylogenetic distance between the donor and receiver species. Finally, we simulated DNA sequences of the coronavirus sequences using the function *simulate_alignment* in HOME. For each type of simulation, we generated 50 simulated datasets of mammal-coronavirus associations. For each dataset, we ran PhyloBayes and ALE on both the mammalian phylogeny and the star phylogeny.
In addition, we used simulations to test for a scenario where coronaviruses originated outside of bats but diversify faster within-bats than within other mammalian lineages, as previously suggested given the efficient immune systems of bats (77). We simulated originations in rodents followed by diversification by preferential host switches (as above) with a host switch rate twice more important in bats (rate 80) than in other mammals (rate 40). For each simulated dataset, we ran PhyloBayes and ALE on the star phylogeny and reported the percentage of originations incorrectly inferred in bats. Parameters of the simulations were chosen to mimick the diversity of the original mammal-coronavirus associations.

Geographic distribution of Coronaviridae

We downloaded geographic range maps for each mammalian host species, with the exception of *Homo sapiens*, from the Map of Life website (https://mol.org/species/); see (68). These maps follow the taxonomy of the Mammal Diversity Database (69) supplemented with the Handbook of the Mammals of the World (HMW) database and the Alien Checklist database for invasive species (68).

We created a world map with hexagonal, equal-area grid cells of 220km on which we mapped host and coronavirus species diversity, using the Mollweide world projection to accurately represent areas. At large spatial scales, cells with ~220km resolution return more reliable diversity

641 estimates than smaller cells (70). We considered that a host species was present in any given cell
642 if its range covered at least 30% of the cell area to avoid overestimating diversity. We calculated
643 host species diversity as a simple sum of the species occurring in any given cell, and host
644 phylogenetic diversity as Faith's phylogenetic diversity index (PD - (71)) for each cell. We mapped
645 Coronaviridae diversity using the host-filling method (72): we constructed a range map for each
646 Coronaviridae sOTU by overlapping the range maps of all its hosts. We consider the host filling
647 method appropriate in this case because coronaviruses are obligatory parasites that can only live
648 inside hosts. Next, we calculated Coronaviridae sOTU diversity by summing range maps
649 overlapping on each cell, and Coronaviridae phylogenetic diversity as Faith's PD (71). We created
650 these maps in R (54) using the packages epm (73), sf (74), and ape (75).
651
652
653 **Acknowledgments**

654 This work was performed using HPC resources from GENCI-IDRIS (Grants 2021-A0100312405
655 and 2022- AD010313735). The authors thank B. Boussau and the Morlon lab for helpful
656 discussions. RM thanks Campus France and the One Health/Make Our Planet Again Program for
657 a fellowship to conduct this research.

References

1. K. P. Alekseev, et al., Bovine-Like Coronaviruses Isolated from Four Species of Captive Wild Ruminants Are Homologous to Bovine Coronaviruses, Based on Complete Genomic Sequences. J Virol 82, 12422–12431 (2008).
2. R. J. de Groot, et al., Family: Coronaviridae. ICTV Ninth Report. https://ictv.global/report_9th/RNApos/Nidovirales/Coronaviridae (2022).
3. A. M. Q. King, Adams M.J., Carstens E.B., Lefkowitz E.J., Virus taxonomy: classification and nomenclature of viruses: Ninth Report of the International Committee on Taxonomy of Viruses (Elsevier, 2012).
4. J. C. Leao, et al., Coronaviridae—Old friends, new enemy! Oral Dis 28, 858–866 (2022).
5. M. Wardeh, M. Baylis, M. S. C. Blagrove, Predicting mammalian hosts in which novel coronaviruses can be generated. Nat Commun 12 (2021).
6. C. M. Zmasek, E. J. Lefkowitz, A. Niewiadomska, R. H. Scheuermann, Genomic evolution of the Coronaviridae family. Virology 570, 123–133 (2022).
7. D. J. Becker, et al., Optimising predictive models to prioritise viral discovery in zoonotic reservoirs. Lancet Microbe 3, e625–e637 (2022).
8. C. Drosten, et al., Identification of a Novel Coronavirus in Patients with Severe Acute Respiratory Syndrome. New England Journal of Medicine 348, 1967–1976 (2003).
9. J. Peiris, et al., Coronavirus as a possible cause of severe acute respiratory syndrome. The Lancet 361, 1319–1325 (2003).
10. A. M. Zaki, S. van Boheemen, T. M. Bestebroer, A. D. M. E. Osterhaus, R. A. M. Fouchier, Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia. New England Journal of Medicine 367, 1814–1820 (2012).
11. P. Zhou, et al., A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579, 270–273 (2020).
12. H. Ritchie, et al., Coronavirus Pandemic (COVID-19). Published online at OurWorldInData.org (2022).
13. P. C. Y. Woo, Y. Huang, S. K. P. Lau, K. Y. Yuen, Coronavirus genomics and bioinformatics analysis. Viruses 2, 1805–1820 (2010).
14. S. J. Anthony, et al., Global patterns in coronavirus diversity. Virus Evol 3 (2017).
15. N. F. R. Munoz, et al., The coevolutionary mosaic of bat betacoronavirus emergence risk. EcoEvoRXiv (2022).
16. Y. Guan, et al., Isolation and characterization of viruses related to the SARS coronavirus from animals in Southern China. Science (1979) 302, 276–278 (2003).
17. C. SMEC, Molecular Evolution of the SARS Coronavirus During the Course of the SARS Epidemic in China. Science (1979) 303, 1666–1669 (2004).
18. R. L. Graham, R. S. Baric, Recombination, Reservoirs, and the Modular Spike: Mechanisms of Coronavirus Cross-Species Transmission. J Virol 84, 3134–3146 (2010).
19. R. J. de Groot, et al., Commentary: Middle East Respiratory Syndrome Coronavirus (MERS-CoV): Announcement of the Coronavirus Study Group. J Virol 87, 7790–7792 (2013).
20. V. M. Corman, D. Muth, D. Niemeyer, C. Drosten, “Hosts and Sources of Endemic Human Coronaviruses” in Advances in Virus Research, (Academic Press Inc., 2018), pp. 163–188.
21. E. Mavrodiev, M. L. Tursky, S. Vincent, D. M. Williams, L. Schroder, On Classication and Taxonomy of Coronaviruses (Riboviria, Nidovirales, Coronaviridae) with Special Focus on Severe Acute Respiratory Syndrome-Related Coronavirus 2 (SARS-CoV-2) (2021) https:/doi.org/10.21203/rs.3.rs-282371/v1.
22. P. C. Y. Woo, S. K. P. Lau, Y. Huang, K. Y. Yuen, Coronavirus diversity, phylogeny and interspecies jumping. Exp Biol Med 234, 1117–1127 (2009).
23. P. C. Y. Woo, et al., 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 (2012).
24. D. Vijaykrishna, et al., Evolutionary Insights into the Ecology of Coronaviruses. J Virol 81, 4012–4020 (2007).
25. A. J. Drummond, S. Y. W. Ho, M. J. Phillips, A. Rambaut, Relaxed Phylogenetics and Dating with Confidence. PLoS Biol 4, e88 (2006).
26. J. O. Wertheim, D. K. W. Chu, J. S. M. Peiris, S. L. Kosakovsky Pond, L. L. M. Poon, A Case for the Ancient Origin of Coronaviruses. J Virol 87, 7039–7045 (2013).
27. J. O. Wertheim, S. L. Kosakovsky Pond, Purifying selection can obscure the ancient age of viral lineages. Mol Biol Evol 28, 3355–3365 (2011).
28. D. T. S. Hayman, M. A. Knox, Estimating the age of the subfamily Orthocoronavirinae using host divergence times as calibration ages at two internal nodes. Virology 563, 20–27 (2021).
29. S. Duchêne, E. C. Holmes, S. Y. W. Ho, Analyses of evolutionary dynamics in viruses are hindered by a time-dependent bias in rate estimates. Proceedings of the Royal Society B: Biological Sciences 281, 20140732 (2014).
30. A. Katzourakis, R. J. Gifford, Endogenous Viral Elements in Animal Genomes. PLoS Genet 6, e1001191 (2010).
31. M. Shi, et al., The evolutionary history of vertebrate RNA viruses. Nature 556, 197–202 (2018).
32. C. Conow, D. Fielder, Y. Ovadia, R. Libeskind-Hadas, Jane: A new tool for the cophylogeny reconstruction problem. Algorithms for Molecular Biology 5 (2010).
33. Y.-Z. Zhang, W.-C. Wu, M. Shi, E. C. Holmes, The diversity, evolution and origins of vertebrate RNA viruses. Curr Opin Virol 31, 9–16 (2018).
34. D. M. de Vienne, et al., Cospeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. New Phytologist 198, 347–385 (2013).
35. B. Perez-Lamarque, H. Morlon, Comparing different computational approaches for detecting long-term vertical transmission in host-associated microbiota in Molecular Ecology, (John Wiley and Sons Inc, 2022) https:/doi.org/10.1111/mec.16681.
36. R. C. Edgar, et al., Petabase-scale sequence alignment catalyses viral discovery. Nature 602, 142–147 (2022).
37. A. Babaian, R. Edgar, Ribovirus classification by a polymerase barcode sequence. PeerJ 10 (2022).
38. B. Perez-Lamarque, et al., Do closely related species interact with similar partners? Testing for phylogenetic signal in bipartite interaction networks. Peer Community Journal 2, XX (2022).
39. G. J. Szöllosi, W. Rosikiewicz, B. Boussau, E. Tannier, V. Daubin, Efficient exploration of the space of reconciled gene trees. Syst Biol 62, 901–912 (2013).
40. G. J. Szöllosi, E. Tannier, N. Lartillot, V. Daubin, Lateral gene transfer from the dead. Syst Biol 62, 386–397 (2013).
41. G. Dudas, L. M. Carvalho, A. Rambaut, T. Bedford, MERS-CoV spillover at the camel-human interface. Elife 7 (2018).
42. C. Wei, et al., Evidence for a mouse origin of the SARS-CoV-2 Omicron variant. Journal of Genetics and Genomics 48, 1111–1121 (2021).
43. S. Santichaivekin, et al., eMPRess: a systematic cophylogeny reconciliation tool. Bioinformatics 37, 2481–2482 (2021).
44. C. C. S. Tan, et al., Transmission of SARS-CoV-2 from humans to animals and potential host adaptation. Nat Commun 13 (2022).
45. N. M. Furey, P. A. Racey, “Conservation Ecology of Cave Bats” in Bats in the Anthropocene: Conservation of Bats in a Changing World, (Springer International Publishing, 2016), pp. 463–500.

46. T. Mildenstein, I. Tanshi, P. A. Racey, “Exploitation of Bats for Bushmeat and Medicine” in Bats in the Anthropocene: Conservation of Bats in a Changing World, (Springer International Publishing, 2016), pp. 325–375.
47. I. Smith, L. F. Wang, Bats and their virome: An important source of emerging viruses capable of infecting humans. Curr Opin Virol 3, 84–91 (2013).
48. S. Leopardi, et al., Interplay between co-divergence and cross-species transmission in the evolutionary history of bat coronaviruses. Infection, Genetics and Evolution 58, 279–289 (2018).
49. M. Pérez-Losada, M. Arenas, J. C. Galán, F. Palero, F. González-Candelas, Recombination in viruses: Mechanisms, methods of study, and evolutionary consequences. Infection, Genetics and Evolution 30, 296–307 (2015).
50. K. Katoh, D. M. Standley, MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Mol Biol Evol 30, 772–780 (2013).
51. S. Capella-Gutiérrez, J. M. Silla-Martínez, T. Gabaldón, trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25, 1972–1973 (2009).
52. R. Bouckaert, et al., BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. PLoS Comput Biol 10, e1003537 (2014).
53. N. Lartillot, H. Philippe, A Bayesian Mixture Model for Across-Site Heterogeneities in the Amino-Acid Replacement Process. Mol Biol Evol 21, 1095–1109 (2004).
54. R Core Team, R: a language and environment for statistical computing (2018).
55. A. Rambaut, A. J. Drummond, D. Xie, G. Baele, M. A. Suchard, Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Syst Biol 67, 901–904 (2018).
56. A. Rambaut, T. T. Lam, L. Max Carvalho, O. G. Pybus, Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). Virus Evol 2, vew007 (2016).
57. T.-H. To, M. Jung, S. Lycett, O. Gascuel, Fast Dating Using Least-Squares Criteria and Algorithms. Syst Biol 65, 82–97 (2016).
58. N. S. Upham, J. A. Esselstyn, W. Jetz, Inferring the mammal tree: Species-level sets of phylogenies for questions in ecology, evolution, and conservation. PLoS Biol 17 (2019).
59. J. Chen, et al., Associating microbiome composition with environmental covariates using generalized UniFrac distances. Bioinformatics 28, 2106–2113 (2012).
60. H. Morlon, et al., RPANDA: an R package for macroevolutionary analyses on phylogenetic trees. Methods Ecol Evol 7, 589–597 (2016).
61. M. Groussin, et al., Unraveling the processes shaping mammalian gut microbiomes over evolutionary time. Nat Commun 8 (2017).
62. M. Bailly-Bechet, et al., How Long Does Wolbachia Remain on Board? Mol Biol Evol 34, 1183–1193 (2017).
63. R. G. Dorrell, et al., Phylogenomic fingerprinting of tempo and functions of horizontal gene transfer within ochrophytes https:/doi.org/10.1073/pnas.2009974118/-/DCSupplemental.
64. C. Barry Cox, The biogeographic regions reconsidered. J Biogeogr 28, 511–523 (2001).
65. B. Perez-Lamarque, H. Morlon, Characterizing symbiont inheritance during host–microbiota evolution: Application to the great apes gut microbiota. Mol Ecol Resour 19, 1659–1671 (2019).
66. D. M., de Vienne, T. Giraud, J. A. Shykoff, When can host shifts produce congruent host and parasite phylogenies? A simulation approach. J Evol Biol 20, 1428–1438 (2007).
67. B. Perez-Lamarque, H. Krehenwinkel, R. G. Gillespie, H. Morlon, Limited evidence for microbial transmission in the phylosymbiosis between Hawaiian spiders and their microbiota. mSystems 7, e01104-21 (2022).
68. C. J. Marsh, et al., Expert range maps of global mammal distributions harmonised to three taxonomic authorities. J Biogeogr 49, 979–992 (2022).
69. C. J. Burgin, J. P. Colella, P. L. Kahn, N. S. Upham, How many species of mammals are there? J Mammal 99, 1-14 (2018).

70. A. H. Hurlbert, W. Jetz, Species richness, hotspots, and the scale dependence of range maps in ecology and conservation. PNAS 104, 13384-13389 (2007).
71. D. P. Faith, Conservation evaluation and phylogenetic diversity. Biol Cons 61, 1-10 (1992).
72. P. Pappalardo, et al., Comparing methods for mapping global parasite diversity. Global Ecol Biogeogr 29, 182-193 (2019).
73. P. O. Title, D. L. Swiderski, M. L. Zelditch, EcoPhyloMapper: an R package for integrating geographical ranges, phylogeny and morphology. Methods Ecol Evol 13, 1912-1922 (2022).
74. E. Pebesma, Simple features for R: standardized support for spatial vector data. The R Journal 10, 439-446 (2018).
75. E. Paradis, K. Schliep, ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35, 526-528 (2019).
76. A.R. Goldberg, et al., Wildlife exposure to SARS-CoV-2 across a human use gradient. BioRXiV, 10.1101/2022.11.04.515237 (2023).
77. A. Banerjee, et al., Novel insights into immune systems of bats. Front. Immunol. 11, 10.3389/fimmu.2020.00026 (2020).

Figures and Tables

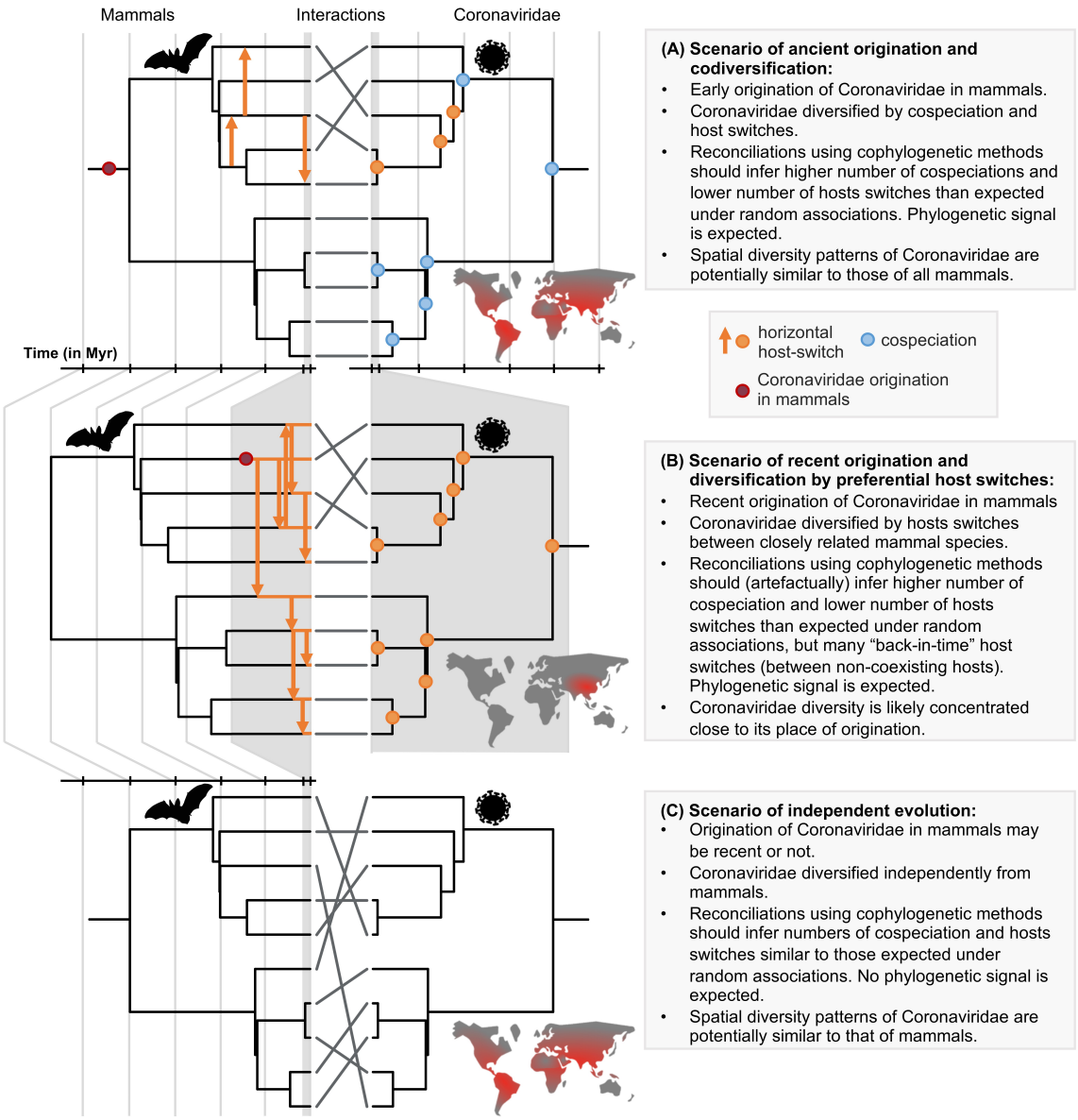

Figure 1. A framework for testing scenarios of virus-host evolution, illustrated with the example of Coronaviridae and their mammalian hosts: In (A), a scenario of ancient origination and codiversification; in (B) a scenario of recent origination and diversification by preferential host switches; and in (C) a scenario of independent evolution. For each scenario, we indicate the associated predictions in the grey boxes. Contrary to scenario C, both scenarios A and B are expected to generate a cophylogenetic signal, *i.e.* closely-related coronaviruses tend to infect closely-related mammals, resulting in significant reconciliations when using topology-based probabilistic cophylogenetic methods, such as the undated version of ALE, Jane, or eMPRess. However, we expect scenario B to be distinguishable from scenario A in terms of the time consistency of host-switching events. Under scenario B, cophylogenetic methods wrongly estimate a combination of cospeciations and "back-in-time" host switches (see Methods & Results). We also

847 expect different biogeographic patterns under the different scenarios, as illustrated by the maps,
848 where the color gradient represents diversity levels (red: high diversity, grey: low diversity).

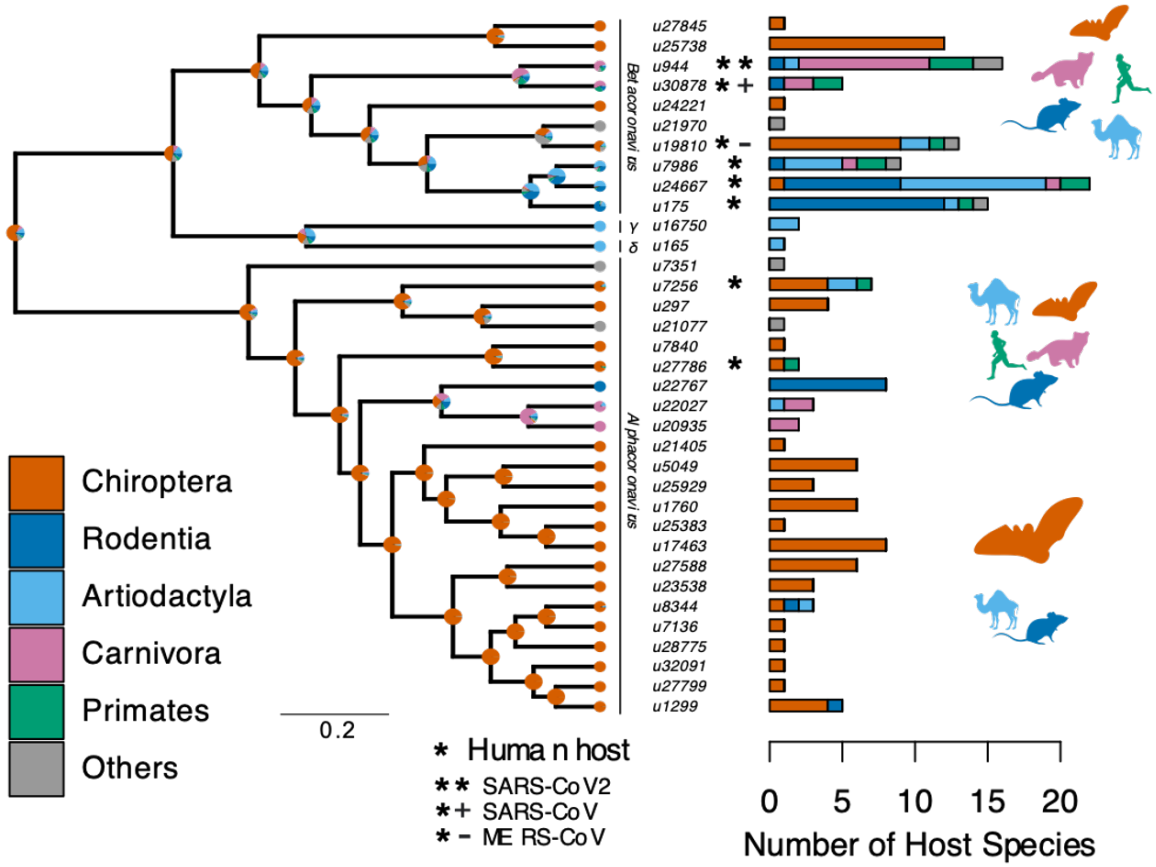

Figure 2. Species-level relationships among coronaviruses and their associated mammalian hosts. A consensus Maximum Clade Credibility phylogenetic tree of coronaviruses is shown on the left. The tree was constructed with BEAST2 based on 150-aa palmprint amino acid sequences of the RdRp gene. sOTUs of Coronaviridae followed the definition of the Serratus project. The putative location of four genera of coronaviruses, Beta, Gamma, Delta, and Alphacoronaviruses, is shown. Bar scale is in units of aa substitution. On the right, a barplot gives the number of total mammalian host species and the number of host species by main mammalian order. Ancestral states on the left were obtained for illustrative purposes with the make.simmap function of the phytools R package (Revell 2012). Mammal silhouettes taken from open-to-use sources in phylopic.org, detailed credits given in SI Appendix Table S6.

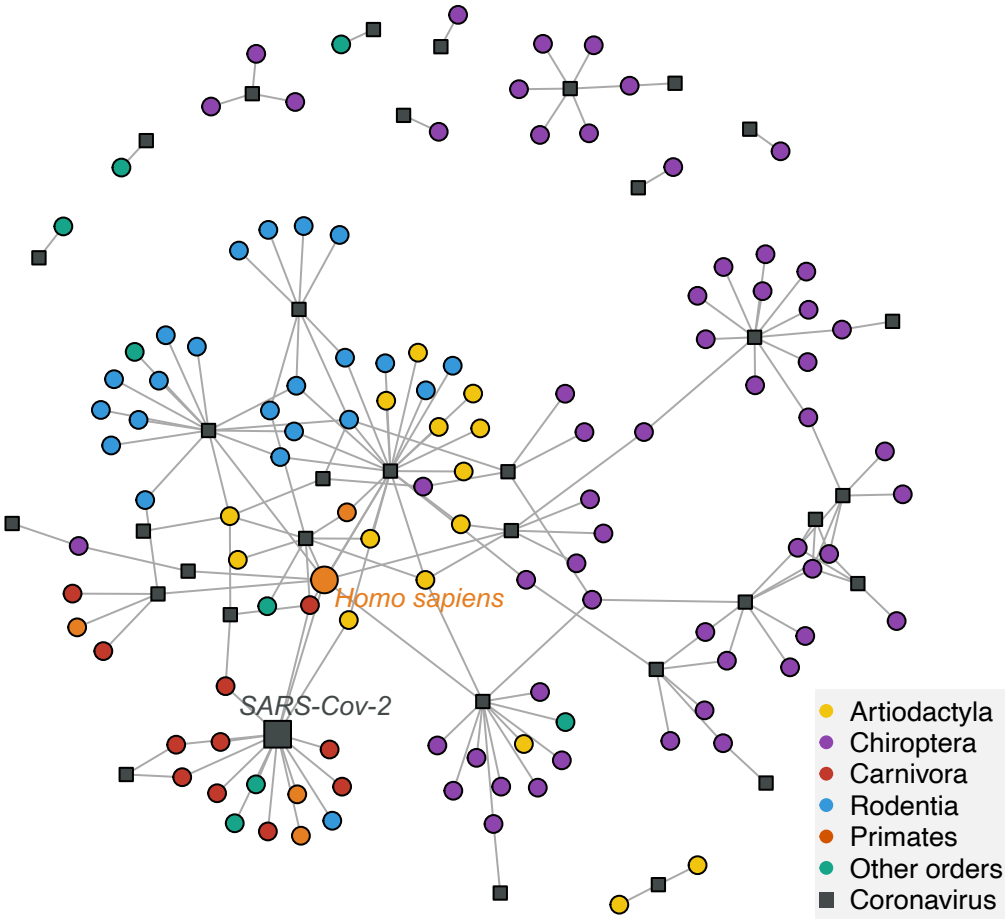

Figure 3. A network visualization of mammal-coronavirus interactions reveals the presence of phylogenetic signal, the isolation of bats, and the centrality of humans. Species-level network representation of the interactions between mammal species and coronavirus sOTUs. Colored round nodes represent mammal species (colors indicate the mammalian order) and grey squared nodes correspond to coronavirus sOTUs. The position of the nodes reflects their similarity in interaction partners, i.e. the tendency of clustering of mammals belonging to the same order can be interpreted as the presence of phylogenetic signal in species interactions. Humans and SARS-Cov-2 are presented using bigger nodes. The plot was obtained using the Fruchterman-Reingold layout algorithm from the igraph R-package.

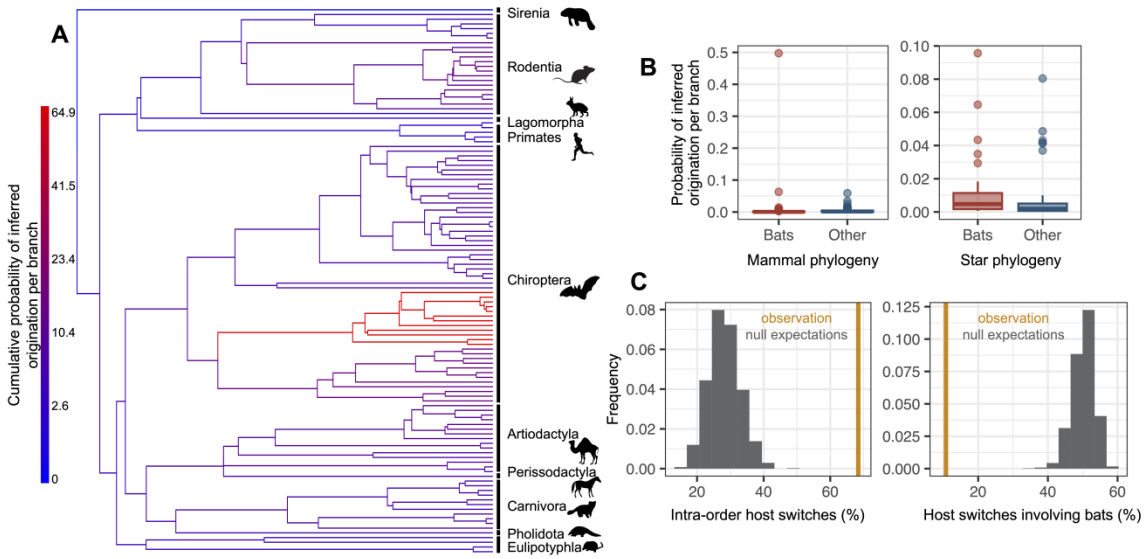

Figure 4. The origination of coronaviruses in mammals is estimated among bats, which tend to form a closed reservoir. (A) Phylogenetic tree of the mammals with branches colored as the percentage of ALE reconciliations which inferred this branch or its ancestral lineages as the origination of coronaviruses in mammals. Red branches are likely originations, whereas blue branches are unlikely. (B) Boxplots recapitulating the probability of inferred origination per branch in bats *versus* other mammal orders, with ALE applied on the original mammal tree (left panel) or on the mammal tree transformed into a star phylogeny (right panel), therefore assuming an origination in extant species. (C) Distributions of the percentages of host switches occurring within mammalian orders (left panel) and between-orders involving bats (right panel). Observed values (in orange) are compared to null expectations if host switches were happening at random (in grey). Mammal silhouettes taken from open-to-use sources in phylopic.org, detailed credits given in SI Appendix Table S6.

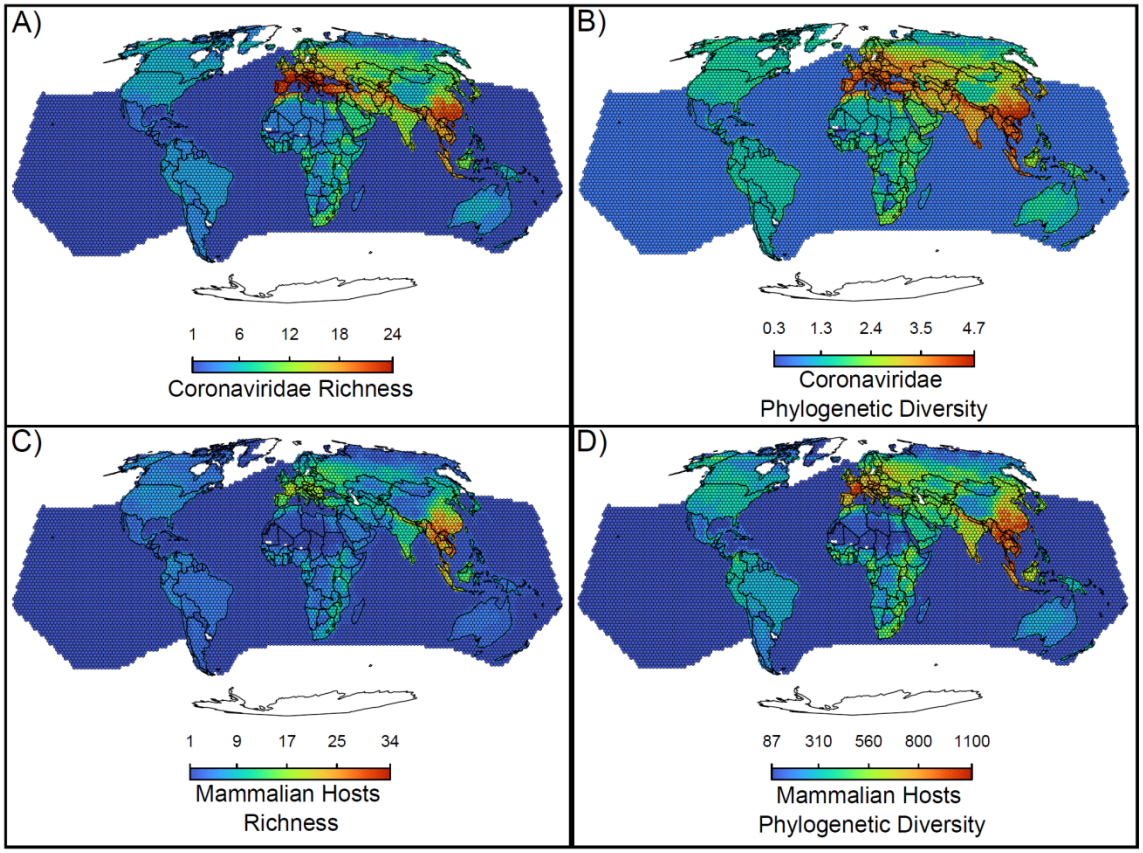

Figure 5. Maps of the diversity of coronaviruses and their mammal hosts. In A) the richness of species of coronaviruses; geographic range maps of coronaviruses were constructed after applying the host-filling method on the geographic range maps of mammalian hosts of coronaviruses. In B) Faith's (1992) phylogenetic diversity of coronaviruses, calculated using the phylogenetic tree of coronaviruses (see main text). In C) and D), the richness and phylogenetic diversity of mammal hosts of coronaviruses, respectively. All maps are on the Mollweide projection.