Raising Concern About Cross-Infection Between Humans and Companion Animals by Evolutionary Analysis of Receptors for SARS-CoV-2

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Abstract

Since the COVID-19 caused by SARS-CoV-2 break out in Wuhan China from Dec. 2019, it has spread to hundreds of countries up to now. Scientists from all over the world have paid tremendous efforts to research and try to control the disease. Previous studies suggested that some of the wild animals could be intermediate hosts between humans and origination of SARS-CoV-2, and some companion animals of humans can be infected by SARS-CoV-2, which raised our curiosity about cross-infection of SARS-CoV-2 between animals and humans. Thus, we select some kinds of animals that might have contact with humans to estimate the susceptibility to SARS-CoV-2 in different animals by evolutionary analysis of their receptors for SARS-CoV-2. The results show that some companion animals of the Felidae family like the cat has a higher infection possibility while the species of the Rodent family like the rat and mouse having close contact with humans show an opposite result, which consist with recent animal experiments and researches. These should raise concerns about cross-infection between human and companion animals or animals having close contact with humans which might turn animals into depositaries of the coronavirus even after control of SARS-CoV-2 spreading and

cause second or more waves of infections after social reopening. Another side of our results stands by the opinion that bioinformatic analysis can be consistent with experiments in some respects so that we can prevent unnecessary sacrifice of laboratory animals in future experiments.

Result

The worldwide breaking out coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 has spread to hundreds of countries with over 3 million cases(1) and attracted more and more scientists' attention. The background of COVID-19 has been stated over and over again by other scientists(2, 3), so we need no repeat in our study.

Like a host of scientists who want to figure out which species transfer the coronavirus to humans from the most possible original virus carrier—bats(4), we initially interested in potential intermediate hosts of SARS-CoV-2. Since previous researches have already found that S protein of SARS-CoV-2 interacts with ACE2 (Angiotensin-converting enzyme 2) of human finally induce the infection of the coronavirus(4-6), we analyze the ACE2 protein sequences (Supplemental Table. Sequences were downloaded from https://www.ncbi.nlm.nih.gov) of different species which partially selected from reported for selling in Wuhan Huanan Seafood market (Supplemental Figure) and others are species from companion animals of humans or having possible contact with humans to find out the potential intermediate host/hosts of SARS-CoV-2. Finally, we find that some of the species we analyzed could be susceptible to SARS-CoV-2 especially some companion animals and domestic animals that have close contact with humans. Because of the possible cross-infection from these animals to humans, we suggest that actions should be taken to prevent the second wave after reopening worldwide.

We find that the phylogenetic tree (Figure 1A) constructing by these selected species is not much as identical as their time tree (Figure 1B) as we expected, especially when we focus on Homo sapiens. This inconsistency caused by different selective pressures, changes in evolutionary rates, or horizontal gene transfer has been mentioned by J. Castresana, L. S. Haggerty, M. Ruvolo, and Hervé Philippe *et al.* (7-10). But in this case, we are more interested in the phylogenetic tree of ACE2 rather than the time tree because the virus was reported interacting with ACE2(4-6). Generally, the phylogenetic tree of ACE2 can sufficiently reveal the susceptibility of species to SARS-CoV-2 if it merely interacts with ACE2 in an organism without any other interference. Yet as we all know our body is a massive system with many unknown signaling pathways so that we cannot take the phylogenetic tree of ACE2 into account only. To further estimate the difference between two type trees of these species, we use the TN (time period plus the number of nodes) method to quantitate the evolutionary distance between *Homo sapiens* and other species selected in the time tree (Supplemental Table. the smaller TN score is the closer evolutionary distance to *Homo sapiens*, the time tree of the species was construct in http://www.timetree.org(11)). Next, we measure the Pairwise distances between ACE2 of *Homo sapiens* and other species by MegaX Software(12) in a phylogenetic tree (Supplemental Table). To compare the results of two types of trees, we sort the species by TN distances (by descending order) and Pairwise distances (also by descending order) and then compare them by ranking scores (Figure 2, Supplemental Table. sort the results of Pairwise distances score minus TN distances score from smallest to largest).

We first speculate that the intermediate hosts may be wild animals that contact humans and transfer the virus to humans by chance. From all species selected we set the cut off as -5 and 5 in our result (Figure 2), which means species scored lower than -5 have much possibility to be intermediate hosts of SARS-CoV-2 (they have less distance against human in the phylogenetic tree than in the time tree) while species scored higher than 5 have contrary results. As our results shown in Figure 2, the species locating at the bottom of histogram should have higher susceptibility to SARS-CoV-2 according to our method. As previous studies reported *Manis javanica*(13) and *Bungarus multicinctu*(14) could be possible intermediate hosts, we find that *Manis javanica* has a low score -7 (Figure 2) which means it might be a possible intermediate host, just like T. T. Lam *et al.* (13) point out that *Manis javanica* has a higher virus carrier rate according to metagenomic analysis. *Manis javanica* could be a possible species who contact both bats and humans so that they can transfer the virus to us because there are still some people who eat *Manis javanica* in China despite the limitation of law. That is why we think it can be a candidate not like another species--snake(14), which is reported could be possible intermediate host locating at the middle of histogram (Figure 2. *Thamnophis elegans*: 2) and we don’t think it has the possibility to be an intermediate host according to our analysis.

Then, when we look through other species locating at the bottom or top of our results histogram. We find that the Felidae family gathering at bottom of the histogram (Figure 2) with lower ranking scores (*Puma concolor*: -30, *Felis catus*: -29, *Lynx pardinus*: -29, *Lynx canadensis*: -26, *Acinonyx jubatus*: -19) than any other species, which means that the Felidae family should be more susceptible than other species we selected. However, another Order of species the Rodent family show contrary results which locating at top of the histogram (Figure

2) with high scores (*Rattus norvegicus*: 16, *Mus musculus*: 16), even though they are closer to humans than the Felidae family is in the time tree. Some subfamily of Rodents like Rats and Mice are considered as the closest model organism to our Primates in laboratories, while this time they lose their status in our analysis. Both of results show above about our analysis are confirmed in experiment recently: J. Shi *et al.* reported that SARS-CoV-2 replicates efficiently in cats(15) and some zoo tigers also reported testing positive for COVID-19, while P. Zhou *et al* show that nCoV-2019 is unable to use mouse ACE2 as an entry receptor in the ACE2-expressing cells(4). Previous study report that the Felidae family diverged earlier than the Rodent family and Primates at chromosome level(16), while our results find that cats have more similar ACE2 sequences with humans than rodents so that the coronaviruses may much easily transfer to cats from humans. This can explain why some early researches find that SARS-CoV-2 antibody could be detected *in vivo* of cats in the pandemic spreading area(17), but cannot infect cells transfected with mouse ACE2 *in vitro*(4). Lynxes are another subfamilies of the Felidae with low scores which not reported getting COVID-19 yet but keep as pets in some country, still, we believe they are susceptible to SARS-CoV-2.

In addition to the species we mentioned above, there are still some of species with not much significant difference between two types of trees but also score upper 5 or lower than -5. Species get lower scores like *Canis lupus familiaris*, *Vulpes vulpes*, and *Paguma larvata* (Figure 2) can be carriers after contact with humans but may slightly affect by SARS-CoV-2 or have mild symptoms. Early study report that SARS-CoV-2 could be poorly detected in dogs(15) but another newly published research reported that there is a dog dying after testing positive for COVID-19(18). These studies showed no evidence that dogs can pass the infection to other dogs or people as cats do, which means dogs might have lower infectivity than cats. One of the moderate scored species--*Paguma larvata* was reported as an intermediate host of another coronavirus SARS-COV (Severe Acute Respiratory Syndrome Coronavirus) in 2003 which has around 80% sequence identity between SARS-CoV-2(4) so *Paguma larvata* may have possibility infected by SARS-CoV-2. Other species got higher numbers above 5 (Figure 2) may have low susceptibility to SARS-CoV-2 like pigs(15) according to our results. These species with not much significant difference may not easy to determine their susceptibilities to SARS-CoV-2 because of their moderate scores.

To further investigate much more detail between SARS-CoV-2 and its receptor ACE2, we choose a particular alpha helix amino acids sequence of ACE2 that is binding with the Receptor Binding Domain (RBD) of SARS-CoV-2(19) and treat this helix sequence with the same method above (Figure 3A). The helix is at the interface between SARS-CoV-2 and ACE2 compound and role as part of the Ligand Binding Domain (LBD) of ACE2 (Figure 3A). Most results are consistent with full length sequence results of ACE2 especially in the Felidae family, they locating at the bottom of the histogram (Figure 3B) which means that their amino acids of alpha helix are similar with human thus they are closer to human than they should be in time tree distances. What's more, we find that *Capra hircus*, *Bos Taurus*, and *Ovis aries* which belong to Suborder Ruminantia have even lower scores than the Felidae family (Figure 3B). So, we speculate that they might also have susceptibilities to SARS-CoV-2 according to our results because they have similar alpha helix sequence with humans that in the interface between SARS-CoV-2 and ACE2.

The result of alpha helix is as identical as part of ACE2 intact sequence analysis, this may because the interface of ACE2 and SARS-CoV-2 contains not only the helix we selected but also some residues from other second structure of ACE2 which we cannot analyses them as a serial sequence combining with the helix by our method. We believe these residues have their important roles through interaction with SARS-CoV-2, so just select a part of the whole protein may merely represent partial respect of full fact. Nevertheless, the single alpha helix also reconfirms our analysis above reminding us about the concern of cross-infection between humans and other animals that might cause the pandemic coming back.

Discussion

Our results reveal that during the worldwide pandemic COVID-19 spreading period, many animals not only pets can catch coronavirus from humans and some of the species that we found with high susceptibilities to the coronavirus are reported being infected yet(15, 17, 18). These species have relatively closer distances to humans in the phylogenetic tree of ACE2 even though they diverge earlier than other species in the time tree. Thus, the coronavirus might transmit to these susceptible species from their owner or after contacting humans. The transmissions of the coronavirus to these species raising our concern even though that infected animals could transmit the coronavirus back to humans or not remains unknown.

In the long term, we suppose that we should not only care about infection between humans and take social distancing guidelines but also need to pay attention to animals around us being possible virus reservoirs that may cause next wave of infection in the future. To prevent further infection caused by the potential reservoirs, we suggest that these actions should be taken from a precautionary point of view: keep distance to your pets if they often play outdoors or reduce the frequencies you bring your pet outdoors; actions should be taken by the governor or community to take care of pets owned by persons who develop SARS-CoV-2 infection instead of cruelly killing them; director of zoo or livestock farm should also take measures to prevent infections of their animals. These steps may avoid potential losses for individuals or communities in the next period of the pandemic.

Another side of our results also reveals that bioinformatic analysis can be consistent with experiments in some respects and guide these experiments before going into detail investigation in order to prevent unnecessary sacrifices of laboratory animals in future experiments.

In conclusion, we find out that some species have higher susceptibilities than other species and might turn into the coronavirus reservoir, not on purpose to motivate people to kill their pets regardless the humanity but reminding them to take action to prevent these risks.

Method

Sequences Data.

All protein sequences were downloaded from https://www.ncbi.nlm.nih.gov and performed with Aliview software(20). All of the species are as follows: *Acinonyx jubatus, Anas platyrhynchos, Aotus nancymaae, Balaenoptera acutorostrata scammoni, Bos taurus, Camelus dromedarius, Canis lupus familiaris, Capra hircus, Carlito syrichta, Chlorocebus aethiops, Cricetulus griseus, Crocuta crocuta, Equus caballus, Erinaceus europaeus, Felis catus, Gallus gallus, Gorilla gorilla gorilla, Heterocephalus glaber, Homo sapiens, Ictidomys tridecemlineatus, Lynx canadensis, Lynx pardinus, Macaca fascicularis, Macaca mulatta, Macaca nemestrina, Manis javanica, Marmota flaviventris, Mesocricetus auratus, Microtus ochrogaster, Mus musculus, Mus pahari, Myotis lucifugus, Nomascus leucogenys, Octodon* *degus, Oryctolagus cuniculus, Ovis aries, Paguma larvata, Pan paniscus, Pan troglodytes, Papio anubis, Physeter catodon, Piliocolobus tephrosceles, Pongo abelii, Puma concolor, Rattus norvegicus, Rhinopithecus roxellana, Sapajus apella, Sus scrofa, Thamnophis elegans, Theropithecus gelada, Urocitellus parryii, Ursus arctos horribilis, Vicugna pacos, Vulpes vulpes.*

Constructing Trees.

The time tree was constructed by online server (http://www.timetree.org) with species listed above. The phylogenetic tree (Neighbor-Joining tree) was constructed by MegaX software(12) (Poisson model, Uniform Rates, Pairwise deletion) using downloaded sequences. Both trees were drawing by iTOL online server (https://itol.embl.de)(21)

Histogram and Diagram.

Histograms of the results were performed using GraphPad Prism software and the diagram of the alpha helix structure was performed by PyMOL software (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.)

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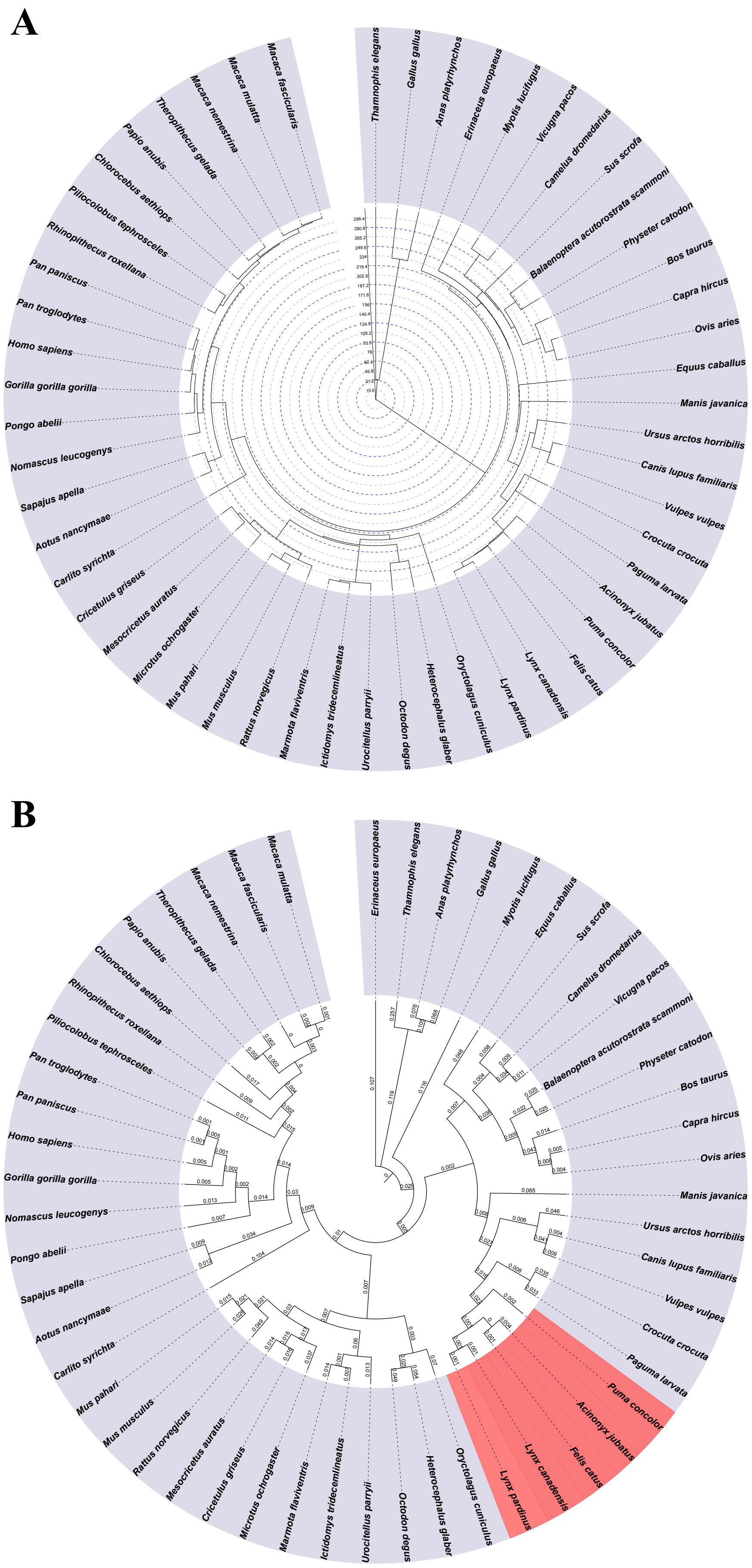
A
Thamnophis elegans
Gallus gallus
Anas platyrhynchos
Erinaceus europaeus
Myotis lucifugus
Vicugna pacos
Camelus dromedarius
Sus scrofa
Balaenoptera acutorostrata scammoni
Physeter catodon
Bos taurus
Capra hircus
Ovis aries
Equus caballus
Manis javanica
Ursus arctos horribilis
Canis lupus familiaris
Vulpes vulpes
Crocuta crocuta
Paguma larvata
Acinonyx jubatus
Puma concolor
Felis catus
Lynx canadensis
Lynx pardinus
Oryctolagus cuniculus
Heterocephalus glaber
Octodon degus
Urocitellus parryii
Ictidomys tridecemlineatus
Marmota flaviventris
Rattus norvegicus
Mus musculus
Mus pahari
Microtus ochrogaster
Mesocricetus auratus
Cricetulus griseus
Carlito syrichta
Aotus nancymaae
Sapajus apella
Nomascus leucogenys
Pongo abelii
Gorilla gorilla gorilla
Homo sapiens
Pan troglodytes
Pan paniscus
Rhinopithecus roxellana
Piliocolobus tephrosceles
Chlorocebus aethiops
Papio anubis
Theropithecus gelada
Macaca nemestrina
Macaca mulatta
Macaca fascicularis
296.4
280.8
265.2
249.6
234
218.4
202.8
187.2
171.6
156
140.4
124.8
109.2
93.6
78
62.4
46.8
31.2
15.6
B
Erinaceus europaeus
Thamnophis elegans
Anas platyrhynchos
Gallus gallus
Myotis lucifugus
Equus caballus
Sus scrofa
Camelus dromedarius
Vicugna pacos
Balaenoptera acutorostrata scammoni
Physeter catodon
Bos taurus
Capra hircus
Ovis aries
Manis javanica
Ursus arctos horribilis
Canis lupus familiaris
Vulpes vulpes
Crocuta crocuta
Paguma larvata
Puma concolor
Acinonyx jubatus
Felis catus
Lynx canadensis
Lynx pardinus
Oryctolagus cuniculus
Heterocephalus glaber
Octodon degus
Urocitellus parryii
Ictidomys tridecemlineatus
Marmota flaviventris
Microtus ochrogaster
Cricetulus griseus
Mesocricetus auratus
Rattus norvegicus
Mus musculus
Mus pahari
Carlito syrichta
Aotus nancymaae
Sapajus apella
Pongo abelii
Nomascus leucogenys
Gorilla gorilla gorilla
Homo sapiens
Pan paniscus
Pan troglodytes
Piliocolobus tephrosceles
Rhinopithecus roxellana
Chlorocebus aethiops
Papio anubis
Theropithecus gelada
Macaca nemestrina
Macaca fascicularis
Macaca mulatta

Figure 1. Time Tree of selected species and Phylogenetic Tree (Neighbor-Joining Tree) of their ACE2. (A) Time tree was constructed by online server (http://www.timetree.org). The Internal tree scale represent the time scale of these species (unit: MYA/Millions of Years Ago). (B) Phylogenetic tree of ACE2 protein sequences. The branch lengths represent the percentage of amino acids mutation in different branches. The Felidae family is label by red leaf ranges.

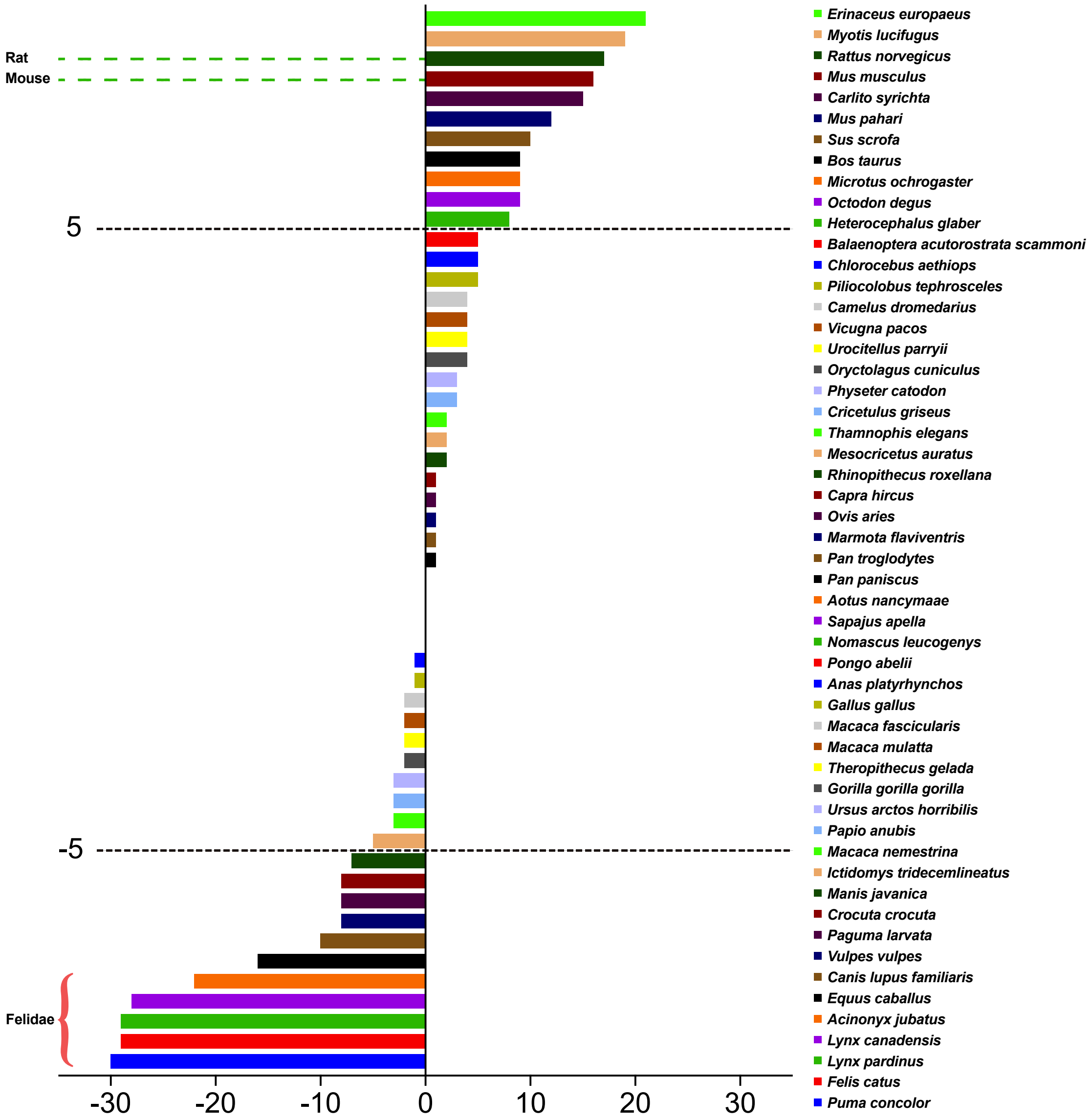

Figure 2. Differences of distances against humans between Time Tree and Phylogenetic Tree of ACE2. Red bracket represent the Felidae family locating at the bottom of the histogram.

Cut-off is set at -5 and 5.

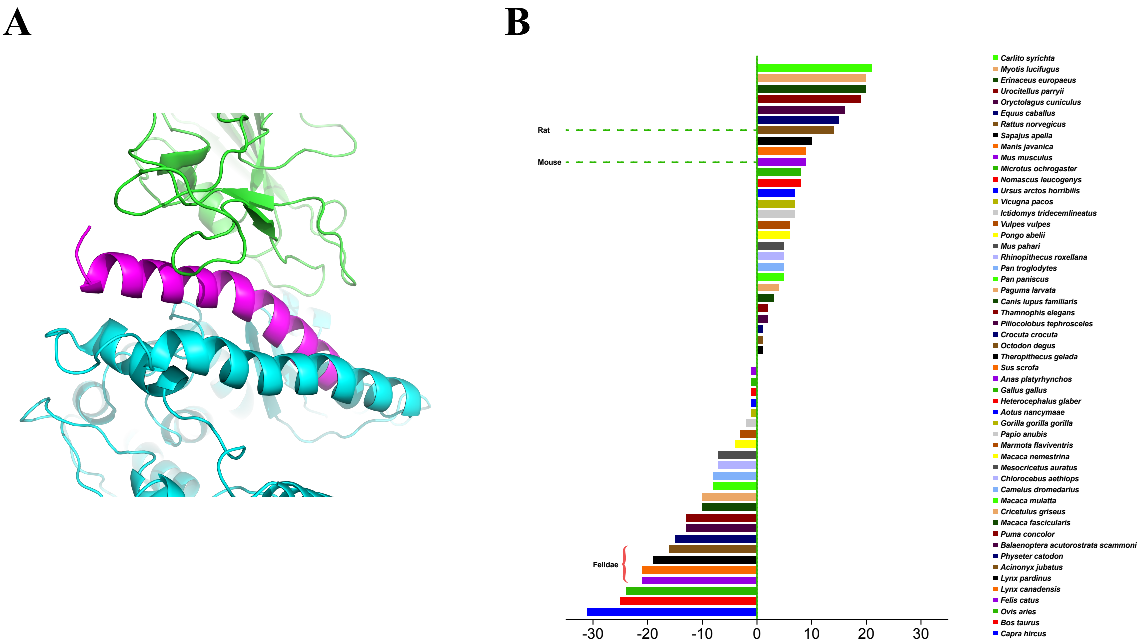

Figure 3. Differences of distances against humans between Time Tree and Phylogenetic Tree of the alpha helix. (A) Diagram of alpha helix in the interface between ACE2 and SARS-CoV-2 (R. Yan et al.)(19). RBD of SARS-CoV-2, the alpha helix of ACE2 and ACE2 colored as green, magenta and cyan respectively.(B) Red bracket represent the Felidae family locating at the bottom of the histogram.