The pangolin's revenge: SARS-CoV-2 did not emerge from a lab but from wildlife exploitation

In recent weeks, news has spread that the current coronavirus is the result of something created in a laboratory that then escaped control. This concern also stems from news reporting that back in 2015 experiments were already being carried out in China to create a recombinant SARS-derived coronavirus. Unfortunately, such news finds fertile ground on social media and, especially in pandemic periods, fosters conspiracies and paranoia. This time, however, the news was spread by some authoritative sources. Here I explain how I found out convincing evidence that SARS-CoV-2 did not emerge from a lab but from wildlife exploitation.

Roberto Cazzolla Gatti

The pangolin's revenge: SARS-CoV-2 did not emerge from a lab but from wildlife exploitation | *GAIA* 29/2 (2020): 79–82
Keywords: coronavirus, COVID-19, environment, epidemic, experiments, pangolin, recombination, wildlife markets

The original recombination experiment

Searching for the origin of SARS-CoV-2 in these days, I found the original report from 2015 published in *Nature Medicine* (Menachery et al. 2015), which allegedly mentioned recombination between the SARS virus injected into mice and the virus SHC014 occurring in the bat *Rhinolophus leschenaultia,* which can make the S proteins (or spikes) that allow the new SARS-CoV-2 virus to adhere to the host cell's receptor (figure 1). I searched for studies in the literature concerning the experiments on recombinant coronavirus SHC014 and the 2015 research by Menachery et al. (2015). Even before reading the full article, I was struck by the curious coincidence that one of the authors had the Key Laboratory of Special Pathogens and Biosafety of the Wuhan Institute of Virology in China as an affiliation. And it is precisely in those laboratories that, as reported in the article, "pseudotyping experiments similar to those that used an HIV-based pseudovirus" (Menachery et al. 2015, in online methods: DOI: 10.1038/nm.3985) were carried out. How likely is it that an unknown virus will spread all over the world and that its starting point is a city where the laboratory was already researching – five years earlier – similar and lethal coronaviruses such as the one that is now causing a worldwide pandemic? Well, my thought was that this would be extremely unlikely.

Nevertheless, at least this time, the fake news was not so fake and conspiracy theorists did have some reason to take it up. If I had stopped my fact check here, I could not have excluded the possibility that what is forcing many humans to quarantine is the result of a genetic manipulation created ad hoc in a laboratory. So I read the 2015 research in full and looked through the supplementary materials seeking any useful elements to better understand what seemed to be a real plot designed against the health of our species. In fact, among the annexes to the article, I found the sequence of the amino acids of S proteins (those of the spikes) useful for the adhesion of coronaviruses to cell receptors. This was the result of genetic engineering between the virus of a bat species and a SARS virus of the mice. The sequence of amino acids, the basic components of proteins that are translated by viral RNA, is extremely important for transmission to humans because it determines the matching with the human angiotensin-converting enzyme receptor type II (more simply ACE2).

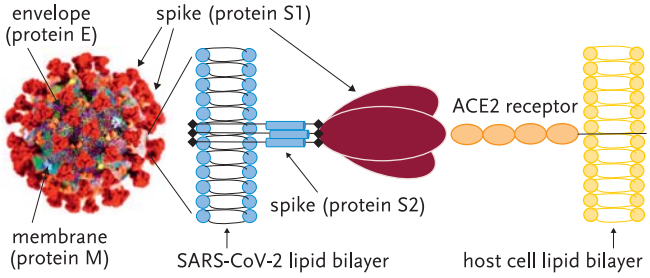

FIGURE 1: The SARS-CoV-2 structure (left) and a magnification of the S1-S2 spike complex used to adhere to the ACE2 receptor of the host cell.

The denial of a lab emergence

After examining the amino acid sequence of the recombined coronavirus, I tried to understand how much truth there was also in the official denial, published by some researchers in a recent letter in the same journal *Nature Medicine* (Andersen et al. 2020). This is taken from the media as proof that the current coronavirus is of natural origin and not created in a laboratory. It was not >

Dr. Roberto Cazzolla Gatti | Konrad Lorenz Institute for Evolution and Cognition Research | Austria *and* Tomsk State University | Biological Institute | Tomsk | Russia | roberto.cazzolla-gatti@kli.ac.at
https://orcid.org/0000-0001-5130-8492

https://doi.org/10.14512/gaia.29.2.3
Submitted March 31, 2020; revised version accepted April 23, 2020 (double-blind peer review).

clear to me, however, if this letter did go through peer-review or if it was published according to the editor's immediate decision. So my suspicion could have continued to exist despite the introduction of the letter read: "Our analyses clearly show that SARS-CoV-2 is not a laboratory construct or a purposefully manipulated virus" (Andersen et al. 2020, p. 450). Although interesting, the comparative analysis proposed by these researchers does not mention the research concerning the coronavirus created in Wuhan in any passage. Moreover, the authors towards the conclusion of the letter admit that "Although the evidence shows that SARS-CoV-2 is not a purposefully manipulated virus, it is currently impossible to prove or disprove the other theories of its origin described here" (Andersen et al. 2020, p. 452).

So, I wanted to understand this issue more clearly: what was the potential evidence for a natural origin of the new SARS-CoV-2 virus that could refute the conspiracy theory?

A comparison of amino acid sequences of the spike proteins

I looked for scientific studies that reported the amino acid sequences of SARS-CoV-2 taken from the first patients hospitalized in Wuhan (Zhou et al. 2020). I tried to compare these sequences with those reported in the letter published by Andersen et al. in *Nature Medicine* (of the SARS-Urbani 2002, of the coronaviruses of mice, bats, and pangolin) and the sequence SHC014 created in the laboratory in 2015. It took some time to match the different positions of the amino acids used in the three studies and create a comparative table, but the result was illuminating (table 1).

What appears quite clearly is that the correspondence of amino acids placed in the key sites for the attachment of the viral spike (S1) to the receptor sites of human cells (marked with an asterisk in table 1) between the current SARS-CoV-2 and the genetically modified coronavirus in 2015 (SHC014) is quite low (one out of six, in position 491). All the other key amino acids are different between the two sequences, as well as between these and the strains of the SARS virus (Urbani strain) that spread in 2002 and the murine one (Mouse-MA15) used as the basis for the engineered coronavirus in 2015. The hypothesis that SARS-CoV-2 was created in, and then escaped from, a laboratory in Wuhan could, therefore, be discredited based on the above comparison.

What emerges, instead, confirmed an equally (or, perhaps, even more) disturbing reality: the sequence of amino acids in the key sites of the S1 protein for adhesion to the ACE2 receptors of SARS-CoV-2 has something similar to that of coronaviruses that infect rhinolophid bats *Rhinolophus affinis* and *Rhinolophus sinicus,* the sequences Bat-RaTG13 (one key amino acid in common in position 442) and Bat-RsWIV1 in table 1 (three key amino acids in common in position 472, 487, and 491), respectively (Zhou et al. 2020, Chen et al. 2020, Benvenuto et al. 2020) and is identical to that of one the most likely intermediate host between bats and humans: the Malayan pangolin, *Manis javanica* (Lam et al. 2020, Li et al. 2020).

Hence, after my facts check, I found a more plausible reason why the SARS-CoV-2 virus could jump over to the first human: the presence of the perhaps largest world market of live animals in the city of Wuhan, where numerous wild and domesticated species are sold, including bats and pangolins.

Pathogen exchange between animals and humans

Wet markets like those in Wuhan (figure 2), where live animals, merchants, and customers crowd together in contaminated and busy roads, make it easier for viruses, like the SARS-CoV-2, to cross from one species to another one, including humans. Where dogs, cats, snakes, raccoons, genets, chickens, pigs, bats, pangolins, fish, seafood, etc. pack in small cages and are slaughtered on the streets, pathogens can find their way to exchange between wild and domesticated animals and humans. Recent examples of cross-species transmission include SARS, which likely emerged from bats, spread to other animals like civet cats, and infected humans in the Guangdong province of southern China in 2002 (Wong et al. 2007); Ebola, which had several outbreaks, with that in 2007 caused by direct exposure to fruit bats of the *Pteropodidae* family in Luebo, Democratic Republic of Congo (Leroy et al. 2009); swine flu, described in April 2009 as a new strain of H1N1 virus, which resulted from a previous triple reassorting of bird, swine, and human flu viruses further combined with a Eurasian pig flu virus (Garten et al. 2009); avian influenza, which is well-known for the HPAI

TABLE 1: Comparison of the spikes' amino acid sequences of different coronavirus strains. Capital letters in the coloured boxes are amino acids; numbers in the first row correspond to the positions of amino acids in the S1 protein's sites for adhesion to the ACE2 receptors. Key sites are marked with an *.

STRAIN	441	442*	472*	473	475	479*	480*	484	486	487*	488	491*
SARS-Urbani (2002)	R	Y	L	N	Y	N	D	Y	T	T	G	Y
Mouse-MA15	R	Y	L	N	Y	N	D	Y	T	T	G	Y
Bat-RsWIV1	R	S	F	N	Y	N	D	Y	T	N	G	Y
Bat-RaTG13	R	L	L	N	Y	Y	R	Y	T	D	G	H
SHC014 (Wuhan 2015)	R	W	P	N	Y	R	P	F	T	A	G	Y
Pangolin-nCoV	R	L	F	N	Y	Q	S	H	T	N	G	Y
SARS-CoV-2 (2020)	R	L	F	N	Y	Q	S	H	T	N	G	Y

strain, H5N1, first appeared in China in 1996 and is most often spread by contact between infected and healthy birds (WHO 2005). Already after the SARS outbreak in 2002, many claimed to close wet markets of live animals in the city of Wuhan and other Chinese municipalities. The markets were temporarily banned, but reopened just after a few months and continued to sell animals until the emergence of this new pandemic.

Moreover, our overexploitation of ecosystems and wildlife (Cazzolla Gatti 2020, Di Marco et al. 2020), has often led to risky contagions: human immunodeficiency viruses (HIV), which was likely transmitted by chimp meat consumption (Hahan et al. 2000, Lemey et al. 2003, Hoppe et al. 2015); simian foamy viruses (SFV), which is an enzootic retrovirus that affects humans bitten by non-human primates (Mouinga-Ondémé et al. 2012) like temple macaques *(Macaca fascicularis)* in Indonesia (Engel et al. 2006); titi monkey adenovirus (TMAdV), which has a high fatality rate (83%) in New World monkeys *(Callicebus cupreus)* and is capable of spreading through human hosts (Chen et al. 2011); malaria and dengue fever, which are more diffused in areas deforested or impacted by climate change (Yasuoka and Levins 2007, Colón-González et al. 2013); meningitis, which can spread faster after prolonged drought (Molesworth et al. 2003).

The emergence of new zoonotic diseases

Something similar likely happened in this pandemic caused by SARS-CoV-2. The Malayan (or Sunda) pangolin, a species at very high risk of extinction according to the International Union for Conservation of Nature[1], is illegally trapped in Southeast Asia, often in those forests where trees are logged to produce tropical timber, paper and palm oil (Wilcove et al. 2013, Cazzolla Gatti et al. 2019). Then, pangolins are sold in Asian wet markets, often together with other critically endangered animals, because they are mistakenly considered to be fundamental elements of traditional medicine or cuisine (Wu et al. 2004). Like other pangolin species, *Manis javanica* is particularly vulnerable to overexploitation due to its very low reproductive output (one, and rarely two, offspring annually), and its populations are critically decreasing in the northern part of its range, such as Laos, extirpated from much of the lowland areas of Myanmar and Thailand, and extremely rare in Vietnam and Cambodia. In Indonesia, although a lack of detailed information on the status of this species, seizures of the last years – involving several thousand animals – indicates that there is intense poaching pressure in the country that is causing population decline (Challender et al. 2019).

Despite all pangolin species, including the Malayan one, are listed by the *Convention on Illegal Trade in Endangered Species of Wild Fauna and Flora (CITES)* in its *Appendix I*[2], which prohibits international trade in wild-caught specimens or their body parts to offer these species the highest level of protection, they are among the most heavily poached protected animals, victims of illegal international trade, largely driven by Asian wildlife markets.

Interestingly, contrary to bats' unique immune system that allows them to harbour many viruses without harming them (Calisher et al. 2006), pangolins seem to have weak immunity and this makes them sensitive to stress and temperature fluctuations (Tang et al. 2019, Wicker et al. 2020). In fact, due to the evolutionary pseudogenization of the interferon epsilon gene[3], innate immunity of >

1 *www.iucnredlist.org/species/12763/123584856*
2 *www.cites.org/eng/app/appendices.php*
3 Interferons are small single-chain glycoproteins, involved in the first line of defence against pathogens such as viruses, bacteria, and parasites.

FIGURE 2: In Asia, wet markets sell live animals including wildlife like snakes, bats, civets and the endangered pangolins trapped illegally in South-east Asian forests.

pangolins seems compromised, resulting in increased susceptibility to infection, particularly in the skin and organs protected by mucus (Choo et al. 2016).

Since pangolins may have intrinsically low mucosal immunity, pathogens might easily infect their lungs, by penetrating lung epithelial cells, and their brain, by penetrating nasal mucosa (Taylor et al. 2010, Dando et al. 2014). Moreover, captive pangolins are prone to frequently fatal pneumonia, gastrointestinal disease, and skin infections (Clark et al. 2008, Hua et al. 2015). Because these species are notoriously difficult to maintain in captivity, the stress and the poor husbandry of wildlife wet markets might render pangolins even more vulnerable to infections by suppressing their immune responses (Choo et al. 2016). In the wild, the interactions with bats might be extremely rare. When these species, however, are forced together in crowded wet markets, where pathogen exposure is rampant, pangolins offer an optimal intermediate host for pathogens and this could have played a fundamental role in this pandemic. Furthermore, climate change and habitat degradation can exacerbate the emergence of new zoonotic diseases because of the immunity sensitiveness for temperature variations and anthropogenic stressors of many wild species and, particularly, of pangolins.

Nonetheless, we cannot blame pangolins because, together with bats and many other animals, they have become – at the same time – victims and executioners of human arrogance. As usual, the abuses perpetrated on nature – sooner or later – come back to haunt us. Will our species ever learn the lessons?

References

Andersen, K. G., A. Rambaut, W. I. Lipkin, E. C. Holmes, R. F. Garry. 2020. The proximal origin of SARS-CoV-2. *Nature Medicine* 26: 450–452.

Benvenuto, D., M. Giovanetti, A. Ciccozzi, S. Spoto, S. Angeletti, M. Ciccozzi. 2020. The 2019-new coronavirus epidemic: Evidence for virus evolution. *Journal of Medical Virology* 92/4: 455–459.

Calisher, C. H., J. E. Childs, H. E. Field, K. V. Holmes, T. Schountz. 2006. Bats: Important reservoir hosts of emerging viruses. *Clinical Microbiology Reviews* 19/3: 531–545.

Cazzolla Gatti, R. 2020. Coronavirus outbreak is a symptom of Gaia's sickness. *Ecological Modelling* 426: 109075.

Cazzolla Gatti, R., J. Liang, A. Velichevskaya, M. Zhou. 2019. Sustainable palm oil may not be so sustainable. *Science of the Total Environment* 652: 48–51.

Challender, D. et al. 2019. *Manis javanica*. *The IUCN red list of threatened species.* DOI: 10.2305/IUCN.UK.2019-3.RLTS.T12763A123584856.en.

Chen, E. C. et al. 2011. Cross-species transmission of a novel adenovirus associated with a fulminant pneumonia outbreak in a new world monkey colony. *PLOS Pathogens* 7/7: e1002155.

Chen, L. et al. 2020. RNA based mNGS approach identifies a novel human coronavirus from two individual pneumonia cases in 2019 Wuhan outbreak. *Emerging Microbes and Infections* 9/1: 313–319.

Choo, S. W. et al. 2016. Pangolin genomes and the evolution of mammalian scales and immunity. *Genome Research* 26/10: 1312–1322.

Colón-González, F. J., C. Fezzi, I. R. Lake, P. R. Hunter. 2013. The effects of weather and climate change on dengue. *PLOS Neglected Tropical Diseases* 7/11: e2503.

Dando, S. J. et al. 2014. Pathogens penetrating the central nervous system: infection pathways and the cellular and molecular mechanisms of invasion. *Clinical microbiology reviews* 27/4: 691–726.

Di Marco, M. et al. 2020. Opinion: Sustainable development must account for pandemic risk. *Proceedings of the National Academy of Sciences* 117/8: 3888–3892.

Engel, G. et al. 2006. Risk assessment: A model for predicting cross-species transmission of simian foamy virus from macaques *(M. fascicularis)* to humans at a monkey temple in Bali, Indonesia. *American Journal of Primatology* 68/9: 934–948.

Garten, R. J. et al. 2009. Antigenic and genetic characteristics of swine-origin 2009 A (H1N1) influenza viruses circulating in humans. *Science* 325/5937: 197–201.

Hoppe, E. et al. 2015. Multiple cross-species transmission events of human adenoviruses (HAdV) during hominine evolution. *Molecular Biology and Evolution* 32/8: 2072–2084.

Lam, T. T. et al. 2020. Identifying SARS-CoV-2 related coronaviruses in Malayan pangolins. *Nature*. DOI. 10.1038/s41586-020-2169-0.

Lemey, P. et al. 2003. Tracing the origin and history of the HIV-2 epidemic. *Proceedings of the National Academy of Sciences* 100/11: 6588–6592.

Leroy, E. M. et al. 2009. Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. *Vector-borne and Zoonotic Diseases* 9/6: 723–728.

Li, C., Y. Yang, L. Re. 2020. Genetic evolution analysis of 2019 novel coronavirus and coronavirus from other species. *Infection, Genetics and Evolution* 10/82: 104285.

Menachery, V. D. et al. 2015. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nature Medicine* 21/12: 1508–1513.

Molesworth, A. M. et al. 2003. Environmental risk and meningitis epidemics in Africa. *Emerging Infectious Diseases* 9/10: 1287–1293.

Mouinga-Ondémé, A. et al. 2012. Cross-species transmission of simian foamy virus to humans in rural Gabon, Central Africa. *Journal of Virology* 86/2: 1255–1260.

Tang, K. N., D. Buoscio, J. Langan, M. J. Adkesson, S. Chinnadurai, C. Aitken-Palmer. 2019. Echocardiographic parameters in african white-bellied pangolins *(phataginus tricuspis)* without cardiac disease. *Journal of Zoo and Wildlife Medicine* 5/3: 604–610.

Taylor, J. B. et al. 2010. Entry of Burkholderia organisms into respiratory epithelium: CFTR, microfilament and microtubule dependence. *Journal of Cystic Fibrosis* 9/1: 36–43.

WHO (Writing Committee of the World Health Organization Consultation on Human Influenza A/H5). 2005. Avian influenza A (H5N1) infection in humans. *New England Journal of Medicine* 353/13: 1374–1385.

Wicker, L. V., K. Lourens, L. K. Hai. 2019. Veterinary health of pangolins. In: *Pangolins. Science, Society and Conservation.* Edited by D. Challender, H. Nash, C. Waterman. Cambridge, MA: Academic. 461–493.

Wilcove, D. S., X. Giam, D. P. Edwards, B. Fisher, L. P. Koh. 2013. Navjot's nightmare revisited: Logging, agriculture, and biodiversity in Southeast Asia. *Trends in Ecology and Evolution* 28/9: 531–540.

Wong, S., S. Lau, P. Woo, K. Y. Yuen. 2007. Bats as a continuing source of emerging infections in humans. *Reviews in Medical Virology* 17/2: 67–91.

Wu, S., N. Liu, Y. Zhang, G. Z. Ma. 2004. Assessment of threatened status of Chinese Pangolin *(Manis pentadactyla)*. *Chinese Journal of Applied and Environmental Biology* 10/4: 456–461.

Yasuoka, J., R. Levins. 2007. Impact of deforestation and agricultural development on anopheline ecology and malaria epidemiology. *American Journal of Tropical Medicine and Hygiene* 76/3: 450–460.

Zhou, P. et al. 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579/7798: 270–273.

Roberto Cazzolla Gatti

Studies in biology, MSc in environmental and evolutionary biology and in global environmental protection. PhD in forest ecology. Associate professor and scientific coordinator of the MSc Program in biodiversity at the Biological Institute of the Tomsk State University, Russia. Research Fellow of the Konrad Lorenz Institute for Evolution and Cognition Research, Austria. Research interests: biodiversity, behavioural ecology, evolutionary and conservation biology, and theoretical/empirical ecology.