





Article

Molecular Detection and Characterization of Coronaviruses in Migratory Ducks from Portugal Show the Circulation of *Gammacoronavirus* and *Deltacoronavirus*

Mahima Hemnani ¹, David Rodrigues ^{2,3} , Nuno Santos ⁴, Sergio Santos-Silva ¹ , Maria Ester Figueiredo ³, Pedro Henriques ², Joana Ferreira-e-Silva ¹, Hugo Rebelo ⁴, Patricia Poeta ^{5,6,7} , Gertrude Thompson ^{1,4} and João R. Mesquita ^{1,8,*} 

- ¹ School of Medicine and Biomedical Sciences, Porto University, 4050-313 Porto, Portugal
 - ² Coimbra College of Agriculture, Polytechnic of Coimbra, 3045-601 Coimbra, Portugal
 - ³ CEF, Forest Research Centre, Edifício Prof. Azevedo Gomes, ISA, Tapada da Ajuda, 1349-017 Lisboa, Portugal
 - ⁴ Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Universidade do Porto, 4485-661 Vairão, Portugal
 - ⁵ Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, Universidade de Trás-os-Montes e Alto Douro (UTAD), 5000-801 Vila Real, Portugal
 - ⁶ Veterinary and Animal Research Centre, Universidade de Trás-os-Montes e Alto Douro (UTAD), 5000-801 Vila Real, Portugal
 - ⁷ Associate Laboratory for Animal and Veterinary Science (AL4 Animals), Universidade de Trás-os-Montes e Alto Douro (UTAD), 5000-801 Vila Real, Portugal
 - ⁸ Epidemiology Research Unit (EPIUnit), Instituto de Saúde Pública da Universidade do Porto, 4050-600 Porto, Portugal
- * Correspondence: jrmesquita@icbas.up.pt



Citation: Hemnani, M.; Rodrigues, D.; Santos, N.; Santos-Silva, S.; Figueiredo, M.E.; Henriques, P.; Ferreira-e-Silva, J.; Rebelo, H.; Poeta, P.; Thompson, G.; et al. Molecular Detection and Characterization of Coronaviruses in Migratory Ducks from Portugal Show the Circulation of *Gammacoronavirus* and *Deltacoronavirus*. *Animals* **2022**, *12*, 3283. <https://doi.org/10.3390/ani12233283>

Academic Editors: Sanjay Rathod and Peirong Jiao

Received: 13 October 2022

Accepted: 23 November 2022

Published: 25 November 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Simple Summary: Migratory birds have an enormous potential for dispersing pathogenic microorganisms. Ducks can host coronaviruses (CoVs), which have a high pathogenic expression and economic impacts, given their ability to migrate exceptional distances, facilitating the dispersal of microorganisms. This study aimed to identify and characterize the diversity of CoVs in migratory ducks from Portugal (*Anas platyrhynchos*, *Anas acuta*, and *Anas crecca*). Among the samples tested, 23 were characterized as *gammacoronavirus* and one as *deltacoronavirus*. The present study aimed to assess the circulation of CoVs in wild ducks from Portugal, being the first description of CoVs for these animals in Portugal.

Abstract: Coronaviruses (CoVs) are part of the *Coronaviridae* family, and the genera Gamma (γ) and Delta (δ) are found mostly in birds. Migratory birds have an enormous potential for dispersing pathogenic microorganisms. Ducks (order Anseriformes) can host CoVs from birds, with pathogenic expression and high economic impact. This study aimed to identify and characterize the diversity of CoVs in migratory ducks from Portugal. Duck stool samples were collected using cloacal swabs from 72 individuals (*Anas platyrhynchos*, *Anas acuta*, and *Anas crecca*). Among the 72 samples tested, 24 showed amplicons of the expected size. Twenty-three were characterized as *Gammacoronavirus* and one as *Deltacoronavirus* (accession numbers ON368935-ON368954; ON721380-ON721383). The *Gammacoronaviruses* sequences showed greater similarities to those obtained in ducks (*Anas platyrhynchos*) from Finland and Poland, *Anas crecca* duck from the USA, and mute swans from Poland. Birds can occupy many habitats and therefore play diverse ecological roles in various ecosystems, especially given their ability to migrate exceptional distances, facilitating the dispersal of microorganisms with animal and/or human impact. There are a considerable number of studies that have detected CoVs in ducks, but none in Portugal. The present study assessed the circulation of CoVs in wild ducks from Portugal, being the first description of CoVs for these animals in Portugal.

Keywords: coronavirus; migratory ducks; *Gammacoronaviruses*; *Deltacoronavirus*

1. Introduction

Coronaviruses (CoVs) belong to the *Coronaviridae* family, subfamily *Orthocoronavirinae*, having a positive-sense single-stranded RNA genome and an envelope equipped with protruding structures on their surface called spikes [1]. Its genome is one of the largest viral RNA genomes, with approximately 25–32 kb [2]. CoVs show a high genetic diversity that can be the result of their large genomes, high rates of mutation, infidelity of the RNA-dependent RNA polymerase, and high frequency of homologous RNA recombination [3].

The *Orthocoronavirinae* subfamily is divided into four genera based on genetic differences and serological cross-reactivity. *Alpha* and *Betacoronaviruses* might have a common ancestor, a CoV originating from bats [4]. For this reason, the viruses belonging to these two genera are found in bats [5] and other mammals, such as swine acute diarrhea syndrome coronavirus (SADS-CoV), transmissible gastroenteritis virus (TGEV), feline coronavirus (FCoV), bovine CoVs (BCoV), and rat coronavirus (RtCoV) [6,7]. On the other hand, *Gamma* and *Deltacoronavirus* evolved from a CoV originating in birds, with the majority of them causing diseases in birds, such as avian CoV infectious bronchitis virus (IBV), turkey CoV (TCoV), goose CoV, and duck CoV [8,9]. Both *Gamma* and *Deltacoronavirus* have been isolated and detected in wild and domestic birds in orders such as Anseriformes, Pelecaniformes, Ciconiiformes, Galliformes, Columbiformes, and Charadriiformes [10–12]. However, *Gammacoronavirus* tends to be detected in domestic birds, while *Deltacoronavirus* infects both domestic and wild birds [13,14]. Two CoVs isolated from cetaceans have also been placed in the genus *Gammacoronavirus* [15,16], and *Deltacoronavirus* has also been found in pigs (porcine deltacoronavirus -PDCoV), causing acute diarrhea and dehydration [17]. Coronaviral infections have received significant attention from both the public and researchers [18].

CoVs have been identified in almost 15 orders of Aves, especially Charadriiformes (seagulls, plovers, sandpipers) and Anseriformes (ducks, geese, swans) [1]. Migratory birds have a huge potential for the transport and dispersal of a large number of pathogenic microorganisms [19]. Ducks, species from the Anseriformes order, can host a number of RNA viruses, including avian CoVs, and avian paramyxovirus type 1, with emerging evidence that suggests they may also be hosts of an array of avian astroviruses [20]. Infections by avian CoVs are characterized by acute, highly contagious, and economically important diseases in domesticated poultry [21]. However, the genetic diversity, evolution, distribution, and taxonomy of some CoVs dominant in birds still remain enigmatic [22]. Therefore, to add knowledge to this specific topic and to understand the role of circulation and the potential transboundary introduction of exotic avian CoVs, this study aimed to identify and characterize the diversity of CoVs in migratory ducks from Portugal.

2. Materials and Methods

2.1. Sample Collection

Samples of duck feces were collected on duck cloaca using cotton swabs and conserved at $-23\text{ }^{\circ}\text{C}$ from ducks captured for marking within duck ecology and migration studies. The species sampled were Mallard *Anas platyrhynchos*, Pintail *Anas acuta*, and Teal *Anas crecca*. Captures were performed in São Jacinto Dunes Nature Reserve (Aveiro) and at EVOA (in Tagus River Estuary Nature Reserve, Vila Franca de Xira) since these are areas of high concentration of ducks where long-term duck ecology studies have been performed (see Figure 1). Ducks were visually marked with nasal saddles and could be followed on the field. A license to capture and mark ducks was obtained from Instituto da Conservação da Natureza e das Florestas (ICNF), Portugal (permit number 40/2021).

Fecal swabs were thoroughly mixed by vortexing in 500 μL of phosphate-buffered saline (PBS) pH 7.2. RNA was extracted from the fecal suspension using the QIAamp viral mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions using 140 μL of the clarified supernatants. Eluted RNA was then kept at $-80\text{ }^{\circ}\text{C}$ until further processing.

compared with the sequences found online in the nucleotide database NCBI (Gen-Bank, Carlsbad, CA, USA).

The viral sequences obtained in this study were submitted to GenBank under the accession numbers ON368935-ON368954 and ON721380-ON721383. These sequences, together with 36 reference strains from the 4 genera (*Alpha*-, *Beta*-, *Gamma*-, and *Deltacoronavirus*) obtained from GenBank, were aligned using MEGA 11 software [24]. Models function on MEGA 11 was used to opt for the model with the smallest Bayesian information criterion (BIC) score [25] using the maximum likelihood method, based on the general time reversible model using a discrete Gamma distribution and assuming evolutionarily invariable sites, 1000 bootstraps replicated, followed by editing with the Interactive Tree of Life (iTOL) platform [26].

3. Results

Among the total 72 samples tested, 24 presented amplicons of the expected size (33.3%; 95% confidence interval [CI]: 22.7–45.4). Bidirectional sequencing of these 24 products, followed by nucleotide BLAST analysis, showed that the majority (n = 23) were characterized as *Gammacoronavirus* (31.4%; 95% CI: 21.4–44.0), and one was characterized as *Deltacoronavirus* (1.4%; 95% CI: 0.03–7.5), as shown in Figure 2. Pintail showed a higher prevalence, with 12 of 24 samples positive (48%). Mallard, a resident species in Portugal (Rodrigues et al. 2000), also had positive samples.

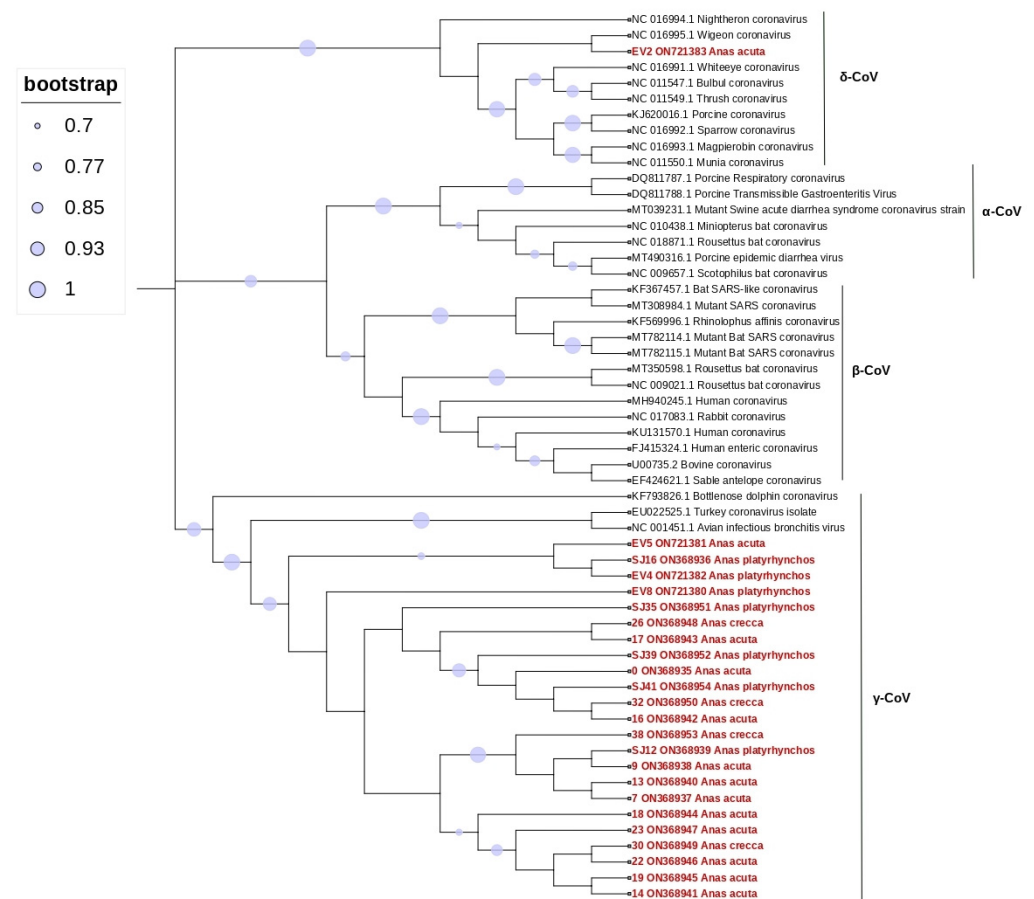


Figure 2. Phylogenetic tree constructed for the *alpha*, *beta*, *gamma*, and *delta* coronavirus, using 36 reference strains and 24 strains identified in this study. Phylogenetic analysis was based on a 406 nt partial region of the RdRp. The tree was constructed using MEGA 10 using the maximum likelihood based on the GTR + G model, and 1000 bootstraps were replicated. Samples from this study are indicated in red.

Sequence analysis within the obtained CoV sequences showed identities ranging from 94% and 100%. Characterization by BLAST indicated that the sequences showed the highest hits (97.76–99.44%) to those sequences obtained from ducks from Finland, Poland, and the USA and mute swans from Poland. Phylogenetic analysis using the obtained 24 CoV sequences and 36 reference strains confirmed the classification as *Gammacoronaviruses* and *Deltacoronavirus* (Figure 2).

The obtained phylogenetic tree showed that the retrieved sequences in this study clustered together with those obtained from a bottlenose dolphin (bottlenose dolphin coronavirus), from a chicken (avian infectious bronchitis virus), and a turkey (turkey coronavirus), with all of them clustering in *Gammacoronavirus*, except for one pintail sample, which clustered with a Wigeon *Anas penelope* (Wigeon coronavirus HKU20) *Deltacoronavirus*. Details of the samples from this study can be found in Table 1.

Table 1. Details of the samples from this study.

Collection Site	Sample ID	Host Species	Accession Number	CoV Genera
Evoa	#0	<i>Anas acuta</i>	ON368935	<i>Gammacoronavirus</i>
	#7	<i>Anas acuta</i>	ON368937	<i>Gammacoronavirus</i>
	#9	<i>Anas acuta</i>	ON368938	<i>Gammacoronavirus</i>
	#13	<i>Anas acuta</i>	ON368940	<i>Gammacoronavirus</i>
	#14	<i>Anas acuta</i>	ON368941	<i>Gammacoronavirus</i>
	#16	<i>Anas acuta</i>	ON368942	<i>Gammacoronavirus</i>
	#17	<i>Anas acuta</i>	ON368943	<i>Gammacoronavirus</i>
	#18	<i>Anas acuta</i>	ON368944	<i>Gammacoronavirus</i>
	#19	<i>Anas acuta</i>	ON368945	<i>Gammacoronavirus</i>
	#22	<i>Anas acuta</i>	ON368946	<i>Gammacoronavirus</i>
	#23	<i>Anas acuta</i>	ON368947	<i>Gammacoronavirus</i>
	#26	<i>Anas crecca</i>	ON368948	<i>Gammacoronavirus</i>
	#30	<i>Anas crecca</i>	ON368949	<i>Gammacoronavirus</i>
	#32	<i>Anas crecca</i>	ON368950	<i>Gammacoronavirus</i>
	#38	<i>Anas crecca</i>	ON368953	<i>Gammacoronavirus</i>
	#EV2	<i>Anas acuta</i>	ON721383	<i>Deltacoronavirus</i>
	#EV4	<i>Anas platyrhynchos</i>	ON721382	<i>Gammacoronavirus</i>
	#EV5	<i>Anas acuta</i>	ON721381	<i>Gammacoronavirus</i>
	S São Jacinto Dunes Nature Reserve	##EV8	<i>Anas platyrhynchos</i>	ON721380
#SJ12		<i>Anas platyrhynchos</i>	ON368939	<i>Gammacoronavirus</i>
#SJ16		<i>Anas platyrhynchos</i>	ON368936	<i>Gammacoronavirus</i>
#SJ35		<i>Anas platyrhynchos</i>	ON368951	<i>Gammacoronavirus</i>
#SJ39		<i>Anas platyrhynchos</i>	ON368952	<i>Gammacoronavirus</i>
#SJ41		<i>Anas platyrhynchos</i>	ON368954	<i>Gammacoronavirus</i>

4. Discussion

The present study assessed the circulation of CoVs in wild ducks from Portugal, being the first description of CoVs for these animals in Portugal. We have also shown that the CoV strains found in the duck population under study are closely related to the *gammacoronavirus* strains retrieved from duck species *Anas platyrhynchos* from Poland and Finland [1,2,27], mute swans from Poland [1], the duck species *Anas crecca* from Hong Kong [11] and the USA (accession number: KJ741882).

As it is known, the *Alphacoronavirus* and *Betacoronavirus* infect mostly mammals, while *gammacoronavirus* and *deltacoronavirus* mainly infect birds [8,9]. While there is a growing number of studies focusing on the presence of coronavirus in bats, there is a variety of CoVs known to circulate among wild and domestic birds as well [2], which, upon entering poultry production premises, may cause severe morbidity and mortality to the birds [28], causing substantial economic losses [29].

Several viruses, including zoonotic and economically significant pathogens, are known to circulate among wild birds [2,30]. The main representative of avian coronavirus is the infectious bronchitis virus (IBV) [31], which is a *Gammacoronavirus*, a highly contagious viral disease that is considered responsible for significant economic losses in the poultry industry worldwide (Moreno et al., 2017). IBV control has been hampered by the intricate IBV evolution over the years, by the emergence of many different antigenic or genotypic types, commonly referred to as variants, that are likely to be facilitated by the spillover through migratory birds [32]; hence, continued monitoring of CoVs in migratory birds is key to mitigating potential outbreaks.

Birds can occupy many habitats and therefore serve diverse ecological roles in various ecosystems [33], especially because of their ability to migrate exceptional distances [34]. The most frequently proposed reason for birds' migration is to benefit from a seasonal availability of resources, to breed, and also to avoid harsh winters [35]. This ability of dispersion can also, unfortunately, facilitate the dispersion of microorganisms with animal and/or human impact [2]. Hence alerts should be made towards surveillance of potentially pathogenic viruses in wild birds.

Gammacoronavirus and *Deltacoronavirus* were detected in a large variety of birds from different countries, such as Sweden [36], Norway [37], England [38], South Korea [39], and Australia [40] in either aquatic or nonaquatic variety, which highlights the potential for viral spillover to domestic species. There is a considerable number of studies that detected CoVs in ducks, but none in Portugal. Ducks are able to occupy diverse ecological niches and are either migratory or resident [1]. The strains of CoV found in our study are closely related to the strains from Finland, and Finnish mallards are strongly migratory [41]. Some species have adapted to urbanized landscapes, increasing their chances of being in contact with humans. The potential for transmissibility between bird species, even between wild and captive members of the same species (e.g., *Anas platyrhynchos*), or between other species, could trigger a captive poultry outbreak, especially in extensively farmed birds, leading to large economic losses in a wide geographical area [42].

Wildlife has been under epidemiological surveillance to identify its possible roles as a reservoir for emerging viruses that may pose a risk to humans and threaten wildlife [21]. Our investigation showed the presence of *Gammacoronavirus* and *Deltacoronavirus* in ducks, indicating that they harbor CoVs and possibly spread it. It is important to continue surveillance of wild ducks for the presence of avian coronavirus to understand better population flyways and ducks' migration, especially the ones with close contact with humans, to understand better the evolution and ecology of coronaviruses, and to monitor emerging new strains that can possibly cause more losses to the poultry industry and transmit to humans [43]. The use of nasal saddles on sampled ducks can also allow the study of possible impacts of CoVs on wild duck species' survival.

5. Conclusions

This study identified *Gammacoronavirus* and *Deltacoronaviruses* in migratory ducks from Portugal, and it is the first report of CoVs for these animals in the country. Migratory birds can occupy many habitats, primarily because of their ability to migrate exceptional distances. This ability of dispersion can also, unfortunately, facilitate the dispersion of microorganisms with animal and/or human impact. Hence, efforts should be made towards the surveillance of potentially pathogenic viruses in wild birds and for monitoring emerging new strains that can possibly cause more losses to the poultry industry and transmit to humans.

Author Contributions: Conceptualization, M.H. and J.R.M.; methodology, M.H., S.S.-S. and J.F.-e.-S.; formal analysis, M.H., D.R., N.S., M.E.F., P.H., H.R., P.P., G.T. and J.R.M.; investigation, M.H., S.S.-S., J.F.-e.-S. and J.R.M.; resources, D.R., N.S. and M.E.F.; sample collection, D.R., N.S. and M.E.F.; writing—original draft preparation, M.H.; writing—review and editing, D.R., N.S., M.E.F., P.H., H.R., P.P., G.T. and J.R.M.; supervision, J.R.M.; project administration, H.R., P.P., G.T. and J.R.M.; funding acquisition, M.H. and J.R.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Fundação para Ciência e Tecnologia (FCT), grant number 2021.09380.BD.

Institutional Review Board Statement: The license to capture and mark ducks was obtained from Instituto da Conservação da Natureza e das Florestas (ICNB), Portugal (permit number 40/2021).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: Mahima Hemnani thanks Fundação para a Ciência e a Tecnologia (FCT) for the financial support of her Ph.D. work under the Maria de Souza scholarship contract number 2021.09380.BD. Sérgio Santos-Silva thanks FCT for the financial support of his Ph.D. work under the Maria de Souza scholarship contract number 2021.09461.BD. This work was financed by national funds through FCT—Fundação para a Ciência e a Tecnologia, I.P., under the projects UIDB/04750/2020 and LA/P/0064/2020. Fieldwork was sponsored by the Association Nationale des Chasseurs de Gibier d’Eau (ANCGE, France) within the project with ESAC « Étude de la migration des canards hivernants au Portugal ».

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Domańska-Blicharz, K.; Miłek-Krupa, J.; Piłkuła, A. Diversity of Coronaviruses in Wild Representatives of the *Aves* Class in Poland. *Viruses* **2021**, *13*, 1497. [[CrossRef](#)] [[PubMed](#)]
2. Hepojoki, S.; Lindh, E.; Vapalahti, O.; Huovilainen, A. Prevalence and genetic diversity of coronaviruses in wild birds, Finland. *Infect. Ecol. Epidemiol.* **2017**, *7*, 1408360. [[CrossRef](#)] [[PubMed](#)]
3. Jackwood, M.W.; Hall, D.; Handel, A. Molecular evolution and emergence of avian gammacoronaviruses. *Infect. Genet. Evol.* **2012**, *12*, 1305–1311. [[CrossRef](#)] [[PubMed](#)]
4. Han, Y.; Du, J.; Su, H.; Zhang, J.; Zhu, G.; Zhang, S.; Wu, Z.; Jin, Q. Identification of Diverse Bat Alphacoronaviruses and Betacoronaviruses in China Provides New Insights Into the Evolution and Origin of Coronavirus-Related Diseases. *Front. Microbiol.* **2019**, *10*, 1900. [[CrossRef](#)]
5. Monchatre-Leroy, E.; Boué, F.; Boucher, J.-M.; Renault, C.; Moutou, F.; Gouilh, M.A.; Umhang, G. Identification of Alpha and Beta Coronavirus in Wildlife Species in France: Bats, Rodents, Rabbits, and Hedgehogs. *Viruses* **2017**, *9*, 364. [[CrossRef](#)]
6. Alluwaimi, A.M.; Alshubaith, I.H.; Al-Ali, A.M.; Abohelaika, S. The Coronaviruses of Animals and Birds: Their Zoonosis, Vaccines, and Models for SARS-CoV and SARS-CoV2. *Front. Veter. Sci.* **2020**, *7*, 582287. [[CrossRef](#)] [[PubMed](#)]
7. Lin, C.-N.; Chan, K.; Ooi, E.; Chiou, M.-T.; Hoang, M.; Hsueh, P.-R.; Ooi, P. Animal Coronavirus Diseases: Parallels with COVID-19 in Humans. *Viruses* **2021**, *13*, 1507. [[CrossRef](#)]
8. Woo, P.C.Y.; Lau, S.K.P.; Lam, C.S.F.; Lau, C.C.Y.; Tsang, A.K.L.; Lau, J.H.N.; Bai, R.; Teng, J.L.L.; Tsang, C.C.C.; Wang, M.; 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.* **2012**, *86*, 3995–4008. [[CrossRef](#)]
9. Cui, J.; Li, F.; Shi, Z.-L. Origin and evolution of pathogenic coronaviruses. *Nat. Rev. Microbiol.* **2019**, *17*, 181–192. [[CrossRef](#)]
10. Muradrasoli, S.; Bálint, Á.; Wahlgren, J.; Waldenström, J.; Belák, S.; Blomberg, J.; Olsen, B. Prevalence and Phylogeny of Coronaviruses in Wild Birds from the Bering Strait Area (Beringia). *PLoS ONE* **2010**, *5*, e13640. [[CrossRef](#)] [[PubMed](#)]
11. Chu, D.K.W.; Leung, C.Y.H.; Gilbert, M.; Joyner, P.H.; Ng, E.M.; Tse, T.M.; Guan, Y.; Peiris, J.S.M.; Poon, L.L.M. Avian Coronavirus in Wild Aquatic Birds. *J. Virol.* **2011**, *85*, 12815–12820. [[CrossRef](#)] [[PubMed](#)]
12. Honkavuori, K.S.; Briese, T.; Krauss, S.; Sanchez, M.D.; Jain, K.; Hutchison, S.K.; Webster, R.G.; Lipkin, W.I. Novel Coronavirus and Astrovirus in Delaware Bay Shorebirds. *PLoS ONE* **2014**, *9*, e93395. [[CrossRef](#)] [[PubMed](#)]
13. Woo, P.C.Y.; Lau, S.K.P.; Lam, C.S.F.; Lai, K.K.Y.; Huang, Y.; Lee, P.; Luk, G.S.M.; Dyrting, K.C.; Chan, K.-H.; Yuen, K.-Y. Comparative Analysis of Complete Genome Sequences of Three Avian Coronaviruses Reveals a Novel Group 3c Coronavirus. *J. Virol.* **2009**, *83*, 908–917. [[CrossRef](#)]
14. Ma, M.; Ji, L.; Ming, L.; Xu, Y.; Zhao, C.; Wang, T.; He, G. Co-circulation of coronavirus and avian influenza virus in wild birds in Shanghai, 2020–2021. *Transbound. Emerg. Dis.* **2022**, 1–7. [[CrossRef](#)]

15. Mihindikulasuriya, K.; Wu, G.; Leger, J.S.; Nordhausen, R.W.; Wang, D. Identification of a Novel Coronavirus from a Beluga Whale by Using a Panviral Microarray. *J. Virol.* **2008**, *82*, 5084–5088. [[CrossRef](#)] [[PubMed](#)]
16. Woo, P.C.Y.; Lau, S.K.P.; Lam, C.S.F.; Tsang, A.K.L.; Hui, S.-W.; Fan, R.Y.Y.; Martelli, P.; Yuen, K.-Y. Discovery of a Novel Bottlenose Dolphin Coronavirus Reveals a Distinct Species of Marine Mammal Coronavirus in Gammacoronavirus. *J. Virol.* **2014**, *88*, 1318–1331. [[CrossRef](#)]
17. Zhang, H.; Han, F.; Shu, X.; Li, Q.; Ding, Q.; Hao, C.; Yan, X.; Xu, M.; Hu, H. Co-infection of porcine epidemic diarrhoea virus and porcine deltacoronavirus enhances the disease severity in piglets. *Transbound. Emerg. Dis.* **2022**, *69*, 1715–1726. [[CrossRef](#)] [[PubMed](#)]
18. Łukaszuk, E.; Dziejulska, D.; Stenzel, T. Occurrence and Phylogenetic Analysis of Avian Coronaviruses in Domestic Pigeons (*Columba livia domestica*) in Poland between 2016 and 2020. *Pathogens* **2022**, *11*, 646. [[CrossRef](#)]
19. Hubálek, Z. An annotated checklist of pathogenic microorganisms associated with migratory birds. *J. Wildl. Dis.* **2004**, *40*, 639–659. [[CrossRef](#)]
20. Wille, M.; Lindqvist, K.; Muradrasoli, S.; Olsen, B.; Järhult, J.D. Urbanization and the dynamics of RNA viruses in Mallards (*Anas platyrhynchos*). *Infect. Genet. Evol.* **2017**, *51*, 89–97. [[CrossRef](#)]
21. Rohaim, M.A.; El Naggar, R.F.; Helal, A.M.; Bayoumi, M.M.; El-Saied, M.A.; Ahmed, K.A.; Shabbir, M.Z.; Munir, M. Genetic Diversity and Phylodynamics of Avian Coronaviruses in Egyptian Wild Birds. *Viruses* **2019**, *11*, 57. [[CrossRef](#)] [[PubMed](#)]
22. Zhuang, Q.-Y.; Wang, K.-C.; Liu, S.; Hou, G.-Y.; Jiang, W.-M.; Wang, S.-C.; Li, J.-P.; Yu, J.-M.; Chen, J.-M. Genomic Analysis and Surveillance of the Coronavirus Dominant in Ducks in China. *PLoS ONE* **2015**, *10*, e0129256. [[CrossRef](#)] [[PubMed](#)]
23. Drzewnioková, P.; Festa, F.; Panzarin, V.; Lelli, D.; Moreno, A.; Zecchin, B.; De Benedictis, P.; Leopardi, S. Best Molecular Tools to Investigate Coronavirus Diversity in Mammals: A Comparison. *Viruses* **2021**, *13*, 1975. [[CrossRef](#)]
24. Tamura, K.; Stecher, G.; Kumar, S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [[CrossRef](#)] [[PubMed](#)]
25. Zhang, D.; Kan, X.; Huss, S.E.; Jiang, L.; Chen, L.-Q.; Hu, Y. Using Phylogenetic Analysis to Investigate Eukaryotic Gene Origin. *J. Vis. Exp.* **2018**, *2018*, e56684. [[CrossRef](#)]
26. Letunic, I.; Bork, P. Interactive Tree Of Life (iTOL) v4: Recent updates and new developments. *Nucleic Acids Res.* **2019**, *47*, 256–259. [[CrossRef](#)]
27. Miłek, J.; Blicharz-Domańska, K. Coronaviruses in avian species—Review with focus on epidemiology and diagnosis in wild birds. *J. Veter- Res.* **2018**, *62*, 249–255. [[CrossRef](#)]
28. Erasmus Medical Centre (NL); Istituto Zooprofilattico Sperimentale delle Venezie (IT); Richard, M.; Fouchier, R.; Monne, I.; Kuiken, T. Mechanisms and risk factors for mutation from low to highly pathogenic avian influenza virus. *EFSA Support. Publ.* **2017**, *14*, 1287E. [[CrossRef](#)]
29. Cavanagh, D. Coronavirus avian infectious bronchitis virus. *Veter- Res.* **2007**, *38*, 281–297. [[CrossRef](#)]
30. Wille, M.; Holmes, E.C. Wild birds as reservoirs for diverse and abundant gamma- and deltacoronaviruses. *FEMS Microbiol. Rev.* **2020**, *44*, 631–644. [[CrossRef](#)]
31. Domanska-Blicharz, K.; Sajewicz-Krukowska, J. Recombinant turkey coronavirus: Are some S gene structures of gammacoronaviruses especially prone to exchange? *Poult. Sci.* **2021**, *100*, 101018. [[CrossRef](#)]
32. Decaro, N.; Lorusso, A. Novel human coronavirus (SARS-CoV-2): A lesson from animal coronaviruses. *Vet. Microbiol.* **2020**, *244*, 108693. [[CrossRef](#)] [[PubMed](#)]
33. Şekercioğlu, H.; Daily, G.C.; Ehrlich, P.R. Ecosystem consequences of bird declines. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 18042–18047. [[CrossRef](#)] [[PubMed](#)]
34. Maurice, M.E.; Fuashi, N.A.; Mbua, R.L.; Mendzen, N.S.; Okon, O.-A.; Ayamba, N.S. The Environmental Influence on the Social Activity of Birds in Buea University Campus, Southwest Region, Cameroon. *Interdiscip. J. Environ. Sci. Educ.* **2020**, *16*, e2210. [[CrossRef](#)]
35. Somveille, M.; Rodrigues, A.S.L.; Manica, A. Why do birds migrate? A macroecological perspective. *Glob. Ecol. Biogeogr.* **2015**, *24*, 664–674. [[CrossRef](#)]
36. Wille, M.; Muradrasoli, S.; Nilsson, A.; Järhult, J.D. High Prevalence and Putative Lineage Maintenance of Avian Coronaviruses in Scandinavian Waterfowl. *PLoS ONE* **2016**, *11*, e0150198. [[CrossRef](#)]
37. Jonassen, C.M.; Kofstad, T.; Larsen, I.-L.; Løvland, A.; Handeland, K.; Follestad, A.; Lillehaug, A. Molecular identification and characterization of novel coronaviruses infecting graylag geese (*Anser anser*), feral pigeons (*Columba livia*) and mallards (*Anas platyrhynchos*). *J. Gen. Virol.* **2005**, *86*, 1597–1607. [[CrossRef](#)]
38. Hughes, L.A.; Savage, C.; Naylor, C.; Bennett, M.; Chantrey, J.; Jones, R. Genetically Diverse Coronaviruses in Wild Bird Populations of Northern England. *Emerg. Infect. Dis.* **2009**, *15*, 1091–1094. [[CrossRef](#)]
39. Kim, H.-R.; Oem, J.-K. Surveillance of Avian Coronaviruses in Wild Bird Populations of Korea. *J. Wildl. Dis.* **2014**, *50*, 964–968. [[CrossRef](#)]
40. Chamings, A.; Nelson, T.; Vibin, J.; Wille, M.; Klaassen, M.; Alexandersen, S. Detection and characterisation of coronaviruses in migratory and non-migratory Australian wild birds. *Sci. Rep.* **2018**, *8*, 5980. [[CrossRef](#)]
41. Gunnarsson, G.; Waldenström, J.; Fransson, T. Direct and indirect effects of winter harshness on the survival of Mallards *Anas platyrhynchos* in northwest Europe. *Ibis* **2012**, *154*, 307–317. [[CrossRef](#)]

42. Blagodatski, A.; Trutneva, K.; Glazova, O.; Mityaeva, O.; Shevkova, L.; Kegeles, E.; Onyanov, N.; Fede, K.; Maznina, A.; Khavina, E.; et al. Avian Influenza in Wild Birds and Poultry: Dissemination Pathways, Monitoring Methods, and Virus Ecology. *Pathogens* **2021**, *10*, 630. [[CrossRef](#)] [[PubMed](#)]
43. Suryaman, G.K.; Soejoedono, R.D.; Setiyono, A.; Poetri, O.N.; Handharyani, E. Isolation and characterization of avian coronavirus from healthy Eclectus parrots (*Eclectus roratus*) from Indonesia. *Veter-World* **2019**, *12*, 1797–1805. [[CrossRef](#)] [[PubMed](#)]