



Surveillance of Coronaviruses in Wild Aquatic Birds in Hong Kong: Expanded Genetic Diversity and Discovery of Novel Subgenus in the Deltacoronavirus

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Background

Gammacoronaviruses (Gamma-CoV) and **Deltacoronaviruses** (Delta-CoV) mainly exist in birds. Wild migratory birds have long-distance migratory capacities and pose a potential risk of spreading disease-causing viruses, resulting in the cross-infection of animals or humans. Surveillance of wild birds to understand the diversity of avian coronaviruses circulating in birds. In this study, we report the findings of coronavirus surveillance conducted on fecal samples of Mai Po aquatic wild birds (N= 3239) from 2018 to 2024. Based on the results of the phylogenetic analysis and DEmARC analysis of the complete gene sequences, we identified two deltacoronaviruses and a gammacoronavirus. They were named **great cormorant coronavirus** (GCCoV, MP18-1070), **black-faced spoonbill coronavirus** (BSCoV, MP22-1474), and **falcated duck coronavirus** (FDCoV, MP22-196), respectively. Of these, BSCoV MP22-1474 was classified into a putative novel subgenus of genus Deltacoronavirus.

Objectives



Understand the diversity of avian coronaviruses circulating in wild birds.



Explore the ecological importance of interspecific transmission of coronaviruses among avian species.

Methodology

Sampling from Mai Po marshes

Extraction by EasyMag/MagNA Pure 96 System

Screening for coronaviruses using pan-coronaviruses reverse transcription-PCR assay targeting the RdRp gene

Confirmation by Sanger Sequencing

Identification of the host species using COX1 gene PCR assay targeting the avian mtDNA

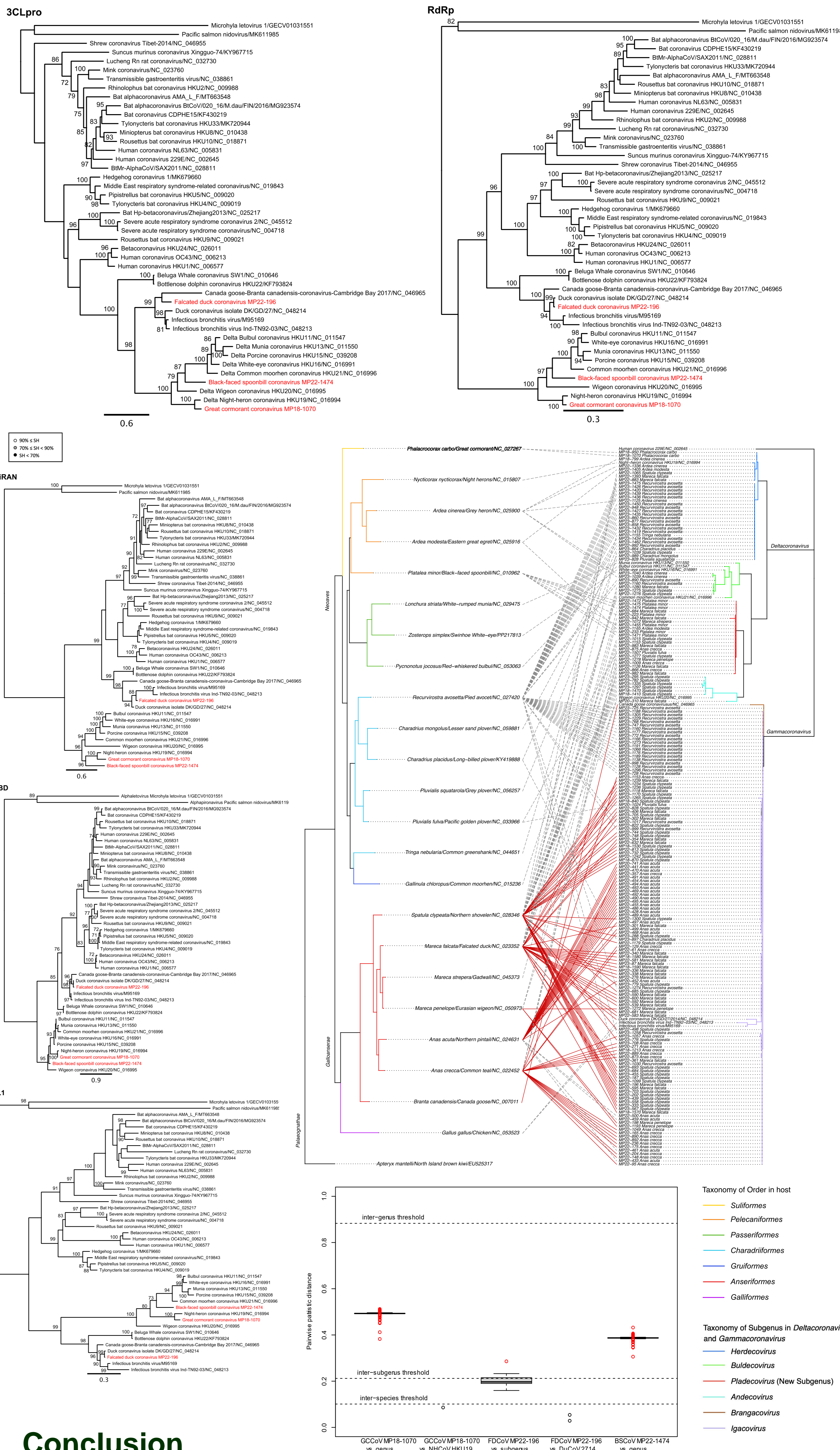
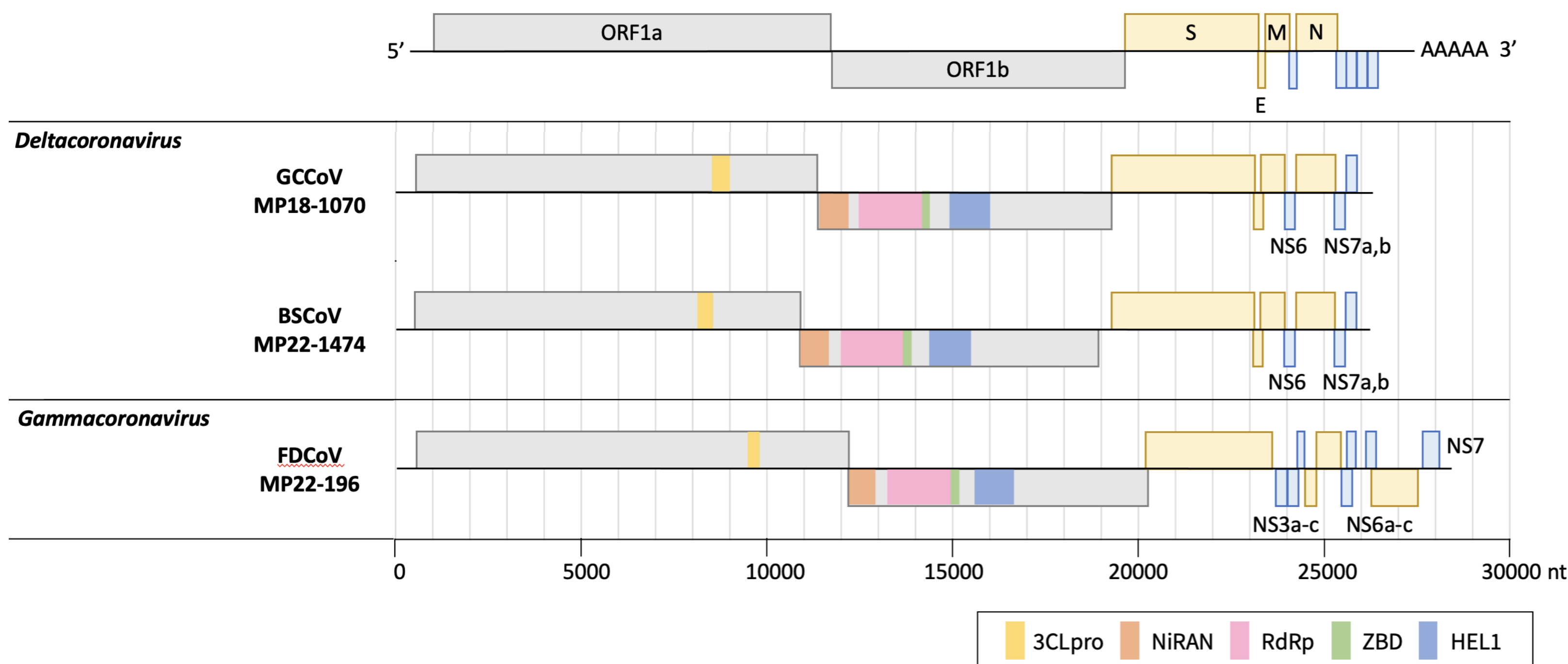
Phylogenetic analysis

Selecting representative novel avian coronaviruses for additional genetic analyses



Results

- The **positive rate** ranged from 5.4% to 16.0%. The host species were identified for 79.8% of the coronavirus-positive samples, with the majority identified in *Anseriformes* order.
- The **prevalence rate** of viruses of the genus Gamma-CoV (64.4%) was higher than that of Delta-CoV (35.6%).
- The genome architecture of these three viruses is similar to other known coronaviruses, with typical genomic organization, predicted transcription regulatory sequences (TRS), and non-structural proteins that are separated by PLpro and 3CLpro cleavage sites.



Conclusion

- Two Delta-CoV with full-genome sequences (GCCoV and BSCoV) and one nearly complete Gamma-CoV genome (FDCoV) were identified in the fecal samples of three bird species.
- Results from phylogenetic analysis and pairwise amino acid identity comparisons using the ICTV classification criteria based on the DEmARC framework, indicate that BSCoV MP22-1474 prototypes a **new subgenus**. GCCoV MP18-1070) and FDCoV MP22-196 belong to two previously known species while diverging most profoundly from known viruses of these species.
- The co-phylogenetic analysis between avian hosts and coronaviruses provides evidence for a **strong linkage** between viruses of the genus *Gammacoronavirus* and the birds of order *Anseriformes*.

References

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