

Fig. 1A

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RbCoV HKU14-1 : CCTTAATTCTCTTACATATTTAATCGACCTAGTTTGGTTGATGTTAATAAGTTTGATGT : 6294
ECoV          : ..... : 6259
PHEV         : A..C.....T.A.....CA.....TCAC....C...A.....A...C... : 6248
BCoV        : ...C.....T.A.....A.....TTA.....AG....A..... : 6248
HCoV OC43   : ...C.....T.A.....A.....TCA.....A.....A..... : 6248

RbCoV HKU14-1 : TTTGAAAGTTGATGATGTTGTTGCAGAAGTTGAATCTTGCAGCTCTGACTTGAGCCATGG : 6354
ECoV          : .....C.....G..A.T.....A.. : 6319
PHEV         : G..A....A.C.....A.A----- : 6271
BCoV        : ...A....G.....AC----- : 6271
HCoV OC43   : ...A....G.....AC----- : 6271

RbCoV HKU14-1 : GAGTTTGAGTGGTAGTGTGAGTACAGGCAGCTCTTACCTGAGCCCACAAGGTAATCAGGG : 6414
ECoV          : ...ACAG..AT.G...C.....A..AT.T.....G...--- : 6376
PHEV         : -----GA..AT..CT.T.A----- : 6290
BCoV        : -----A...G...AT.TCT..A----- : 6290
HCoV OC43   : -----A...G...AC..CT..A----- : 6290

RbCoV HKU14-1 : GAGTAATGTCGAAGCTCATACTGACTGTTCTTCTGCTTCAGGTAATCAAGGGAGTAATGCAAT : 6474
ECoV          : .G...C.....TG.G.TG...C.T.G...C...C.....T....GC : 6436
PHEV         : ...GC...A...A-----CA..AAA.A.C----- : 6318
BCoV        : ...G...C.A..A-----TC...A.A.A.C----- : 6318
HCoV OC43   : ...GG..C.A...A-----C...A.A.A.C----- : 6318

RbCoV HKU14-1 : TAATGGTTCAGCTAATAGTAATAAGATTGTAAATTAATGGTGTAAAAAGCCTTTTAA : 6534
ECoV          : .....T...C.T...C.....T..... : 6496
PHEV         : -----C..C...C.G.....G.....A..... : 6356
BCoV        : -----C..A...G...G.....A..A..... : 6356
HCoV OC43   : -----C..A...G...G.....A..A..... : 6356

RbCoV HKU14-1 : GGTTGAAAATAGTGTGTTGTTGTTAATGACAGCACTAGTGAGACCAAATTTGTTAAGAGTTT : 6594
ECoV          : .....A....A.....T.....T..... : 6556
PHEV         : .....G.....C...A.....TGA.....C.....A.....C.. : 6416
BCoV        : .....G.....CA.A.....TGAT.....A.T...A..... : 6416
HCoV OC43   : .....G.....CA.....TGAT.....A.....A..... : 6416

RbCoV HKU14-1 : GTCTATTGTTGATGTTTATGATATGTGGCTGACTGGTAGTCGGTATGTTGTTAA : 6648
ECoV          : .....G.....T..... : 6610
PHEV         : .....G.....T.A...T..AA..... : 6470
BCoV        : .....A.....G.....T..A...T..A.....G : 6470
HCoV OC43   : .....G.....T.A...T..AA.....G : 6470
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Fig. 1B

RbCoV HKU14-1 : ATTTACAGCACTGACTTTGGATAAAGTTCCTT-----CCAAATTGCAGTGTAC : 18080  
ECoV : .....C.....C----- : 18039  
PHEV : G.....TA..T...C..A.....G..ACAGGCCGTCGAAA.G..G.T..A...G : 17914  
BCoV : G.....TA..T...C..A.....G..ACAGGCCGTTGAAA.G.G.G.T..A...G : 17914  
HCoV OC43 : G.....TA..T...C..A.....G..ACAGGCCGTCGAAA.T..G.T..A...G : 17914

RbCoV HKU14-1 : TACCAATTTATTCAAAGATTGTAGCAAGAGCTATATTGGTTACCATCCAGCTCATGCTCC : 18140  
ECoV : ..T.....T.....G.....C..... : 18099  
PHEV : ..T.....T.....AT...G.....C.....T..... : 17974  
BCoV : ..T.....T.....G.....C.....C..... : 17974  
HCoV OC43 : ..T.....T.....T...GC...T..C..... : 17974

RbCoV HKU14-1 : TTCATTTTTAGCAGTGGATGAAAAATACAAGGTAATGGTGATTTGGCCGTTTGCCTTGG : 18200  
ECoV : ..G.....G.....T.....T..... : 18159  
PHEV : .....G.....A.....C.....T.....CT.C..C....A.....G..T..... : 18034  
BCoV : .....G.....A.....C.....T.....C..C...C....A.....G..T..... : 18034  
HCoV OC43 : .....G.....A.....C.....T.....C..C...C....A.....G..T..... : 18034

RbCoV HKU14-1 : AGTAGGTGATTCCTCTGTAACATATCTAGACTAATATCACTCATGGGTTTTAAGTTAGA : 18260  
ECoV : .....A..... : 18219  
PHEV : TA.TA.....TG...T.....A...T.....AC.G.. : 18094  
BCoV : TA.T.A.....TG...T.....A...T.....AC.T.. : 18094  
HCoV OC43 : TA.T.....TG...T.....A...T.....A..G.. : 18094

RbCoV HKU14-1 : TCTGACACTTGAGGGATATTGTAAGCTATTTATTACAAAAGAAGAGGCTGTTAAACGTGT : 18320  
ECoV : .T.....T.....T.....C..... : 18279  
PHEV : .G.T..C....C..G.....T.....A..T.....A.....C.. : 18154  
BCoV : .G.C..C....T..G.....T.....A..T.....A.....C.. : 18154  
HCoV OC43 : .G.T..C....T..G.....T.....A..T.....A.....C.. : 18154

RbCoV HKU14-1 : TAGAGCTTGGGTCGGCTTCGACGCTGAAGGTGCCACGCTACACGTGACAACATTGGAAC : 18380  
ECoV : .....A.....T..G.....C..T..T...G.....A..G.. : 18339  
PHEV : GC.T.....T.....T..T.....T..T..C..G.....T.G.....G.. : 18214  
BCoV : GC.T.....T.....T..T.....C..T..T..C..G.....T.G.....G.. : 18214  
HCoV OC43 : GC.T..C....T.....T..T.....T..T..C..G.....T.G.....G.. : 18214

RbCoV HKU14-1 : TAATTTCCCCTTACAATTAGGATTTTCCACTGGAATAGATTTTGTAGTGAAGCTACTGG : 18440  
ECoV : ..C.....AC.T.....G.....T.....C..... : 18399  
PHEV : A.....AC.T.....G.....A.....T.....T.....C..... : 18274  
BCoV : A.....AC.T.....G.....T..A.....T.....C..... : 18274  
HCoV OC43 : A.....AC.T.....A.....T.....T.....C..... : 18274

RbCoV HKU14-1 : TTTGTTTGCAGAAAGAGATGGTTACAGTTTGAAGAAAGCTGTGGCCAAAGCTCCACCTGG : 18500  
ECoV : .....CT.T..G.....A..G.....G.....A.. : 18459  
PHEV : .....T..T.....C..A..G.....T.....T..... : 18334  
BCoV : .....T..T.....C..A..G.....T.....T..... : 18334  
HCoV OC43 : .....T..T.....C..A..G.....G.....T..... : 18334  
RbCoV HKU14-1 : TGAACAATTTAAACATCTCATCCCGTTAATGTCCAGGGACAGCGGTGGGATGTTGTTAG : 18560  
ECoV : C.....G..C..T.....T..G..A.TAGA..T..A..A..... : 18519  
PHEV : .....G..C.....T..G..A.GAGA..T...C..... : 18394  
BCoV : .....G.....T..G..A.GAGA..T...C..... : 18394  
HCoV OC43 : .....G..C.....T..G..A.GAGA..T..T..C..... : 18394

RbCoV HKU14-1 : ACCTAGAATAGTACAAATGTTTTTCAGATCATTTAGTTGATCTGGCTGATAGCGTTGTA : 18620

ECoV : .....T..... : 18579  
 PHEV : .....G.....A.....T.....T.T.....G.. : 18454  
 BCoV : .....G.....A.....T.....T.T.....G.. : 18454  
 HCoV OC43 : .....G.....A.....T.....T.T.....G.. : 18454

RbCoV HKU14-1 : TGTTACATGGGCTGCCAGCTTTGAGCTTACTTGTCTCCGATATTTTGCGAAAATAGGTAA : 18680  
 ECoV : .....C.....C.....T..GT..... : 18639  
 PHEV : A.....G.....A.....C..A.....T..C....TA...G.T..ACG : 18514  
 BCoV : A.....A.....A.....C.....C.....A...G.....CG : 18514  
 HCoV OC43 : A.....A...A.....C.....C..C.....A...G....GCG : 18514

RbCoV HKU14-1 : GGAGACATGTTGTAATGTGTGCACTAATCGTGCCACAGTCTATAATTCTAGAACTGGTTA : 18740  
 ECoV : .....T.....T.....C.....T..... : 18699  
 PHEV : T...TT.....A.....T..C..... : 18574  
 BCoV : T...TC.C.....A.....CT..C..... : 18574  
 HCoV OC43 : T...TT.C.....A.....A.....T..C..... : 18574

RbCoV HKU14-1 : CTATGGCTGCTGGCGTCATAGTGTTT : 18766  
 ECoV : ..... : 18725  
 PHEV : .....T..T.....C.....A : 18600  
 BCoV : .....T..T.....C.....A : 18600  
 HCoV OC43 : .....T..T.....C.....A : 18600

Fig. 1C

RbCoV HKU14-1 : TTGTATTATAATTTACCTGCTGCTAATGTTTCTGTTAGCAGATTAAATCCTTCTACCTGG : 25133  
BCoV : .....G..T.....T... : 24984  
HCoV OC43 : .....G..T.....T... : 25017  
PHEV : C.C.....G..G.....A..A.....A.....C.CTCAT.AT.....AT.T... : 24770  
ECoV : ..A.....G..GGG.....A.....A.....C.C..CT.....G..... : 25087

RbCoV HKU14-1 : AATAGAAGGTTTGGTTTTACAGAGCAATCTGTTTTTAAGCCTCAGCCTGCAGGTGTTTTTC : 25193  
BCoV : .....G..A.....A.....A.....T.....T : 25044  
HCoV OC43 : ...AG..A.....T...AG.T.....GA.....C.T : 25077  
PHEV : .....A..G.....---AT...A.AG..TG.TTC...-----CC.--- : 24818  
ECoV : .....GC...A.....---TG.TA.AA.G.....T...T : 25144

RbCoV HKU14-1 : ACTGCTCATGATGTTGTCTATGCACAACATGTTTTAAAGCTCCACACTTTCTGTCCA : 25253  
BCoV : .....A.....T.....T.....A.A.....G : 25104  
HCoV OC43 : ...AA.....A..T.....C.....C.....T.AA.A.....G : 25137  
PHEV : -----C...T...T...G..A.....TA.A..T.A..A.AT.....C : 24872  
ECoV : ...AAC.....G..T..T..A...G..C.....G.T..T.A..A.AT..C..T : 25204

RbCoV HKU14-1 : TGCAAATTGGATGGTTCACTTTGTGTTGGTAGTGGCTCTGGTGTGATGCCGGTTTTAA : 25313  
BCoV : ..T.....G..TT.G.....A...A..TC.....A..A.....T...A... : 25164  
HCoV OC43 : ..T.....A.....---TTCG...A.....TC.....A----- : 25178  
PHEV : ..T.G.ACAAG.CA-----A..CA.A..G...---A..ACA.GAA.TT..CC.GT. : 24923  
ECoV : ..T..GAACA.....G..ACC.....A..C.A...TAT.TC..C..GA.T.A..GG..CT : 25264

RbCoV HKU14-1 : CATACTGGTATAGGCACTTGCCCTGCAGGTACTAATTATTTAACAATGTTATAATTCTGTT : 25373  
BCoV : AC..G...C.....T.....T...CC.....G...CC : 25224  
HCoV OC43 : A...A.....T.....T...G..... : 25232  
PHEV : GGA..CAC.G.GC.T.AG..TTT...T.CAGT..C.A.CGCT..TAAG.G..C----- : 24977  
ECoV : ACC.....CTCT.....T...T.....ATC...CGC..T...T...C..A.A : 25324

RbCoV HKU14-1 : CAATGTAACGTGTTCAATGTACACCAGATCCTATTT---TATCTAAAGCTACAGGCCCTAT : 25430  
BCoV : .....G..T...TTG..C..C.....C..C..A--C.....G..T..C : 25281  
HCoV OC43 : -----TTG..C..T..T.....A--C..T.....TA.T... : 25278  
PHEV : -----CTGG..CCA.....TCCA--C..A.....G.GT.AATG.T.GG : 25025  
ECoV : .....GCA..CACT...GAT..T..G.....AATGC.AT.TT.A.CT.T...TCG. : 25384

RbCoV HKU14-1 : AAGTGCCCCCAAACAACTAAGTATTTAGTTGGTGTAGGTGAGCATGTTCCGGTCTTGCTATT : 25490  
BCoV : .....A.....CA.....C..... : 25341  
HCoV OC43 : .....A.C.....CA.....C.....G... : 25338  
PHEV : .CT..T..G...T.C...AGT..CTA.ACAAC...C.....CC.T...T.G.G.C... : 25085  
ECoV : GCT..T..T...GTC...C.C.T.....C.T..A.....C..A...T.A.GC..A : 25444

RbCoV HKU14-1 : AAAAGTGATTATTTGTTGGAGGCAATCCTTGTACTTGCCAACCACAAGCTTTTTTGGGTTGG : 25550  
BCoV : .....C.....T.....A..... : 25401  
HCoV OC43 : .....T.....G.....A..... : 25398  
PHEV : GTGGAG...G...CTCT...C.....C.....TA.....G..CA.A.C... : 25145  
ECoV : C.TGAA...T..C.....TCA.....T...T.C.G.CA.T..C..A..C... : 25504  
RbCoV HKU14-1 : TCTGTGGATTCTTGTTTACAAGGAGACAGGTGTAATATTTTGGCTAATTTAATTTTGCAT : 25610  
BCoV : .....T..C.....G..T.....T..... : 25461  
HCoV OC43 : .....CA..C.....A.....T..... : 25458  
PHEV : AGCTCA..AA.....G...AAT.GT.....T.....A... : 25205  
ECoV : ....CA.....AT.GAC.C..C.....T.C.....AA.. : 25564

RbCoV HKU14-1 : GGTGTTAATAGTGGTACTACTTGTCTACTGATTTACAAAAGCTAACACAGATATAATT : 25670

BCoV : .A.....T.A.....C..... : 25521  
HCoV OC43 : .A.....CT.....C..... : 25518  
PHEV : .A.....C.....A.C.....C.G.G...T.TA...T.C. : 25265  
ECoV : .....G.....CGGT.C...T..T..G..CG.. : 25624

RbCoV HKU14-1 : GTTGGTGTGTTGTTAATTATGATCTCTATGGCATCTCAGGCCAAGGCATTTTTGTGCGAG : 25730  
BCoV : C.....T.....T..TA.T.....T.....T... : 25581  
HCoV OC43 : C.....C.....T..T.T.....T... : 25578  
PHEV : AC..A.....C..A.....TA.....G.....AC..A.A..A : 25325  
ECoV : .....A.....CT.G..C..T..TA...T.....A.T... : 25684

RbCoV HKU14-1 : GTTAATGCGACTTATTATAATAGTTGGCAGAACCTTTTATATGATTCTAATGGTAATCTC : 25790  
BCoV : ..... : 25641  
HCoV OC43 : ..... : 25638  
PHEV : .....C.....T...C.T.....G..... : 25385  
ECoV : .....C.....CC.T....C..... : 25744

RbCoV HKU14-1 : TATGGTTTTAGAGACTACTTAACAAATAGAACTTTTATGATTCGTAGTTGCTATAGCGGT : 25850  
BCoV : .....T... : 25701  
HCoV OC43 : ..C.....A.....C..... : 25698  
PHEV : .....C.....T..T..T.....C.T.....C.....T..A : 25445  
ECoV : .....G..T...C.T..T.....T.....T... : 25804

RbCoV HKU14-1 : CGTGTTCAGCGCCTTTCACGCTAATCTTCCGAACCAGCATTGCTATTTCCGAATA : 25908  
BCoV : .....T.....C..... : 25759  
HCoV OC43 : .....T.....C..... : 25756  
PHEV : A.A.....A.TT.....T.....C.....T.....T...A.G.....T...C : 25503  
ECoV : A.A.....C..T.AC...T..G..G.A..T..G.....TC.TT.G.A...T...T : 25862

### **Legends to Supplementary Figure**

Supplementary FIG. 1. Multiple alignments of sequences from representative regions of RbCoV HKU14, BCoV, PHEV, ECoV and HCoV OC43 genomes upstream or downstream to potential recombination sites. Sequences corresponding to positions 6235 to 6648 (A), 18033 to 18766 (B) and 25074 to 25908 (C) of strain RbCoV HKU14-1 were used. Identical bases between RbCoV HKU14 and the most closely related viruses were highlighted.

Supplementary Table 1. Primers used for RT-PCR amplification of the leader-body junction of subgenomic mRNAs (sg mRNAs) of RbCoV HKU14

| Primer   | Sequence (5'-3')         | Use                                 |
|----------|--------------------------|-------------------------------------|
| LPW18462 | GATTCTGAGCGATTGCGTGC     | Forward primer for all sg mRNAs PCR |
| LPW18463 | GTAAACCTTTATAATTTAACACA  | Reverse primer for mRNA (NS2a) PCR  |
| LPW18464 | AATCCGTGAAGTGGAAGCTC     | Reverse primer for mRNA (HE) PCR    |
| LPW18465 | CCATAGTGTGCTCAATAGTAGAGT | Reverse primer for mRNA (S) PCR     |
| LPW18466 | GTGTTAGCGAATGCTGTGAAAC   | Reverse primer for mRNA (NS5a) PCR  |
| LPW18467 | CTCATACAACTGCCTACCTCT    | Reverse primer for mRNA (E) PCR     |
| LPW18468 | CCAAGATACACATTATTCAACG   | Reverse primer for mRNA (M) PCR     |
| LPW18469 | GAGTAATTCCAGAGAACCAAGA   | Reverse primer for mRNA (N) PCR     |

Supplementary Table 2. Comparison of amino acid identities of the seven conserved replicase domains [ADRP, nsp5 (3CL<sup>pro</sup>), nsp12 (RdRp), nsp13 (Hel), nsp14 (ExoN), nsp15 (NendoU) and nsp16 (O-MT)] of RbCoV HKU14 and Betacoronavirus 1 for species demarcation

| CoV           | Domains            | RbCoV HKU14-1 | ECoV | PHEV | HCoV OC43 | BCoV |
|---------------|--------------------|---------------|------|------|-----------|------|
| RbCoV HKU14-1 | ADRP               | -             | 97.5 | 84   | 82.4      | 83.2 |
|               | 3CL <sup>pro</sup> | -             | 92.7 | 92.1 | 91.7      | 92.7 |
|               | RdRp               | -             | 95   | 95.3 | 94.6      | 95.5 |
|               | Hel                | -             | 98.8 | 96.4 | 96.2      | 96.4 |
|               | ExoN               | -             | 96.9 | 91.7 | 91.2      | 91.6 |
|               | NendoU             | -             | 85.6 | 88.3 | 88.8      | 88   |
|               | O-MT               | -             | 95.3 | 97.3 | 97        | 97   |

Domains sharing < 90% identity are highlighted in grey

Supplementary Table 3. Estimation of non-synonymous substitution and synonymous rates in the four genomes of RbCoV HKU14

|        | Ka    | Ks    | Ka/Ks |
|--------|-------|-------|-------|
| orf1ab | 0.003 | 0.007 | 0.429 |
| nsp1   | 0.001 | -     | -     |
| nsp2   | 0.002 | 0.005 | 0.400 |
| nsp3   | 0.002 | 0.008 | 0.250 |
| nsp4   | -     | 0.010 | -     |
| nsp5   | 0.001 | 0.008 | 0.125 |
| nsp6   | -     | 0.023 | -     |
| nsp7   | -     | 0.020 | -     |
| nsp8   | 0.001 | 0.003 | 0.333 |
| nsp9   | -     | -     | -     |
| nsp10  | 0.002 | 0.009 | 0.222 |
| nsp11  | -     | -     | -     |
| nsp12  | 0.002 | 0.013 | 0.154 |
| nsp13  | 0.000 | 0.100 | 0.000 |
| nsp14  | 0.005 | 0.023 | 0.217 |
| nsp15  | 0.003 | 0.009 | 0.333 |
| nsp16  | -     | 0.018 | -     |
| HE     | 0.006 | 0.024 | 0.250 |
| S      | 0.003 | 0.017 | 0.176 |
| NS5a   | 0.002 | -     | -     |
| E      | 0.008 | -     | -     |
| M      | 0.002 | 0.014 | 0.143 |
| N      | 0.001 | 0.100 | 0.010 |