**Supplementary Material**

**Virologica Sinica**

**The Continued Evolution of the H10N3 Influenza Virus with Adaptive Mutations Poses an Increased Threat to Mammals**

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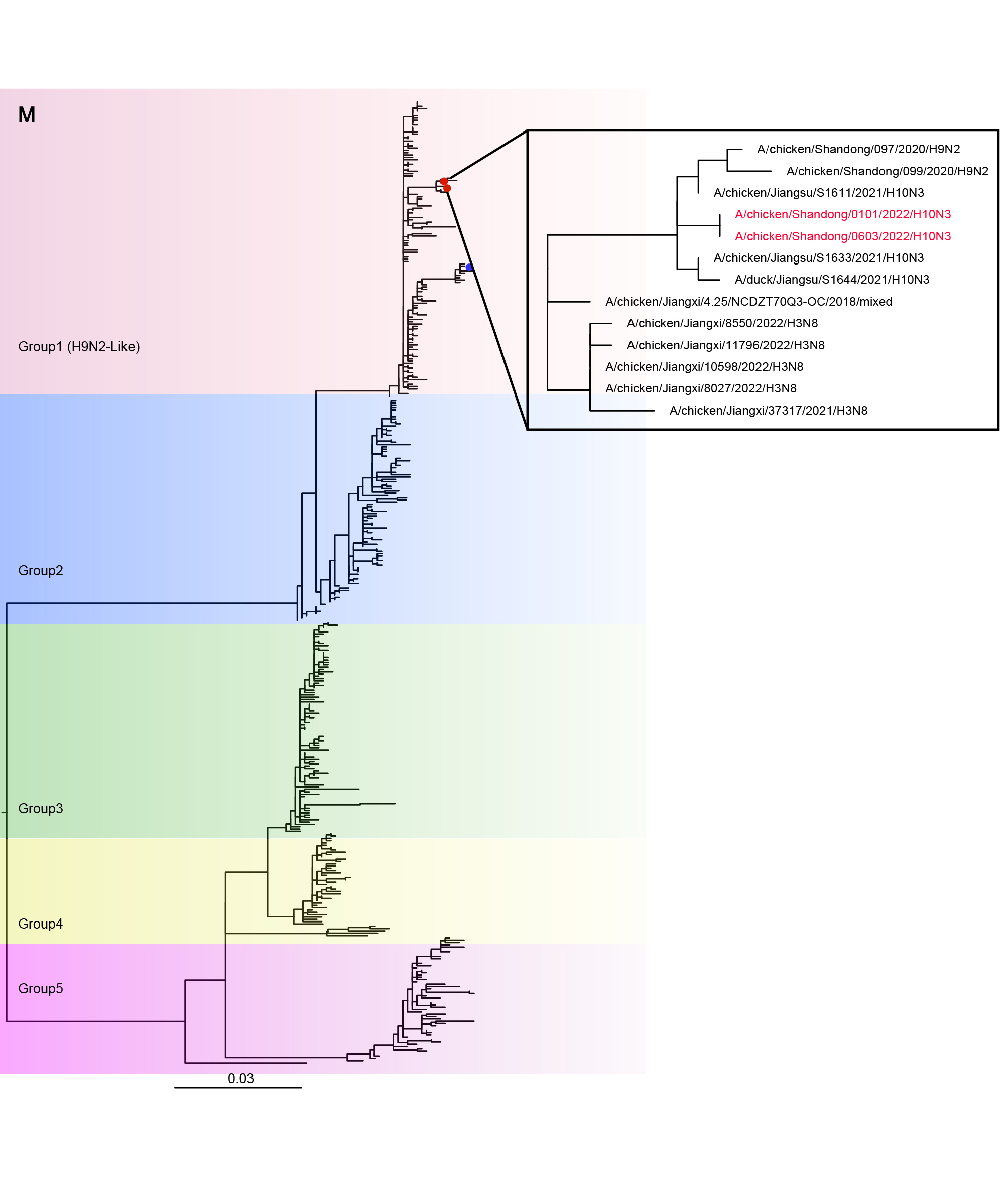
**1** Shiping Ding, Jiangtao Zhou contributed equally to the authors.

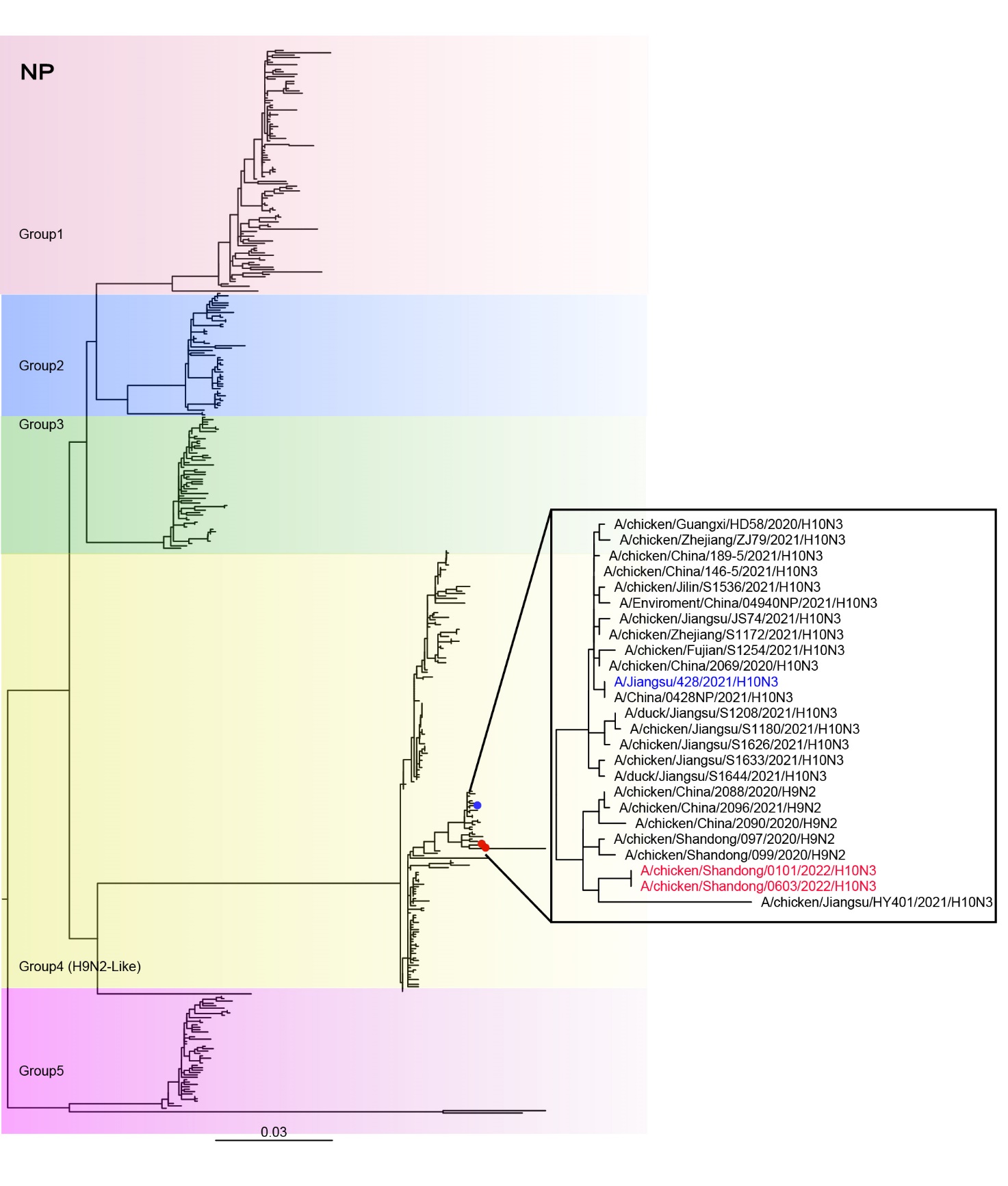
\***Correspondence**:

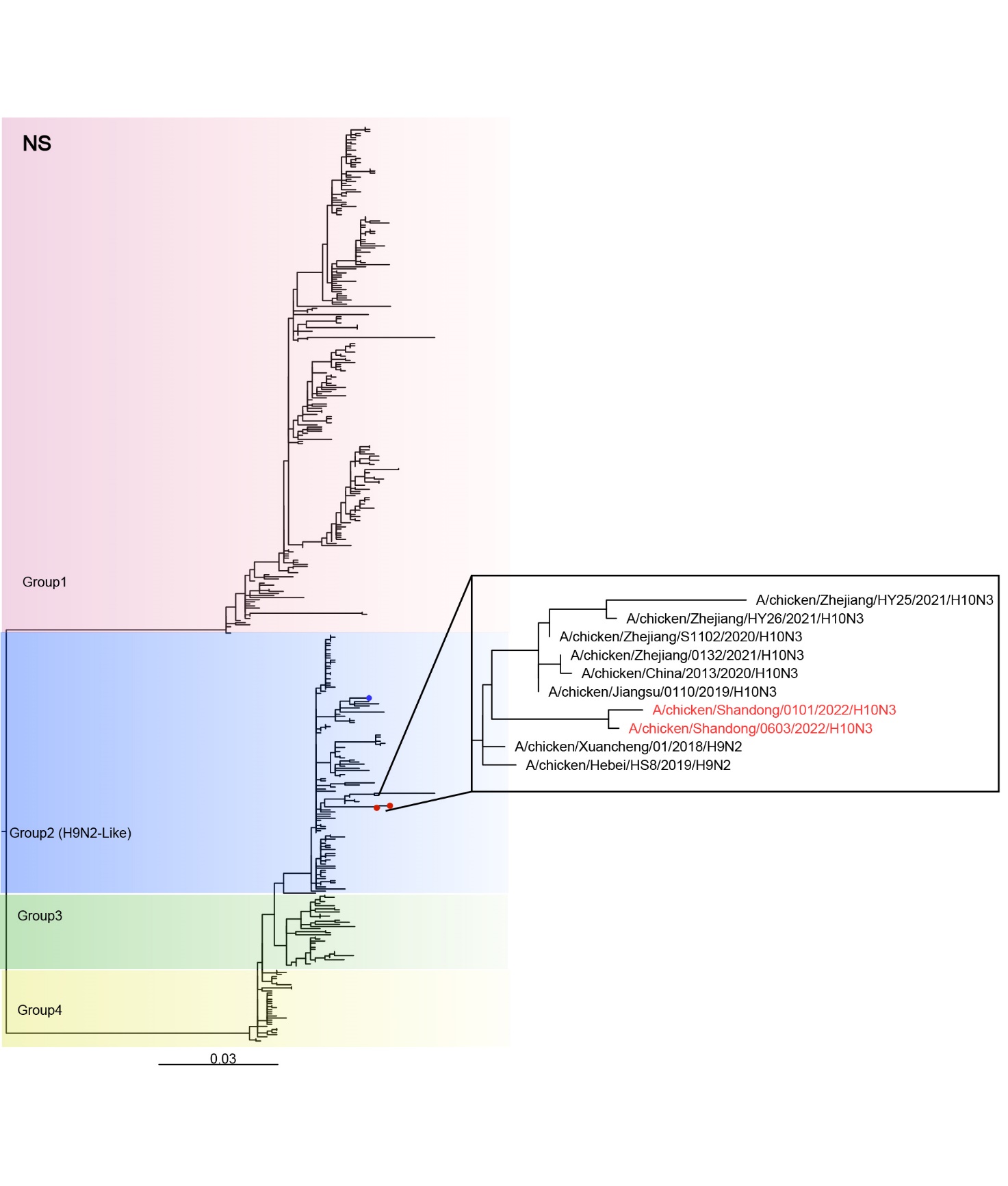
E-mail addresses: qiwenbao@scau.edu.cn (W. Qi); jiahaozhang@hzau.edu.cn (J. Zhang)

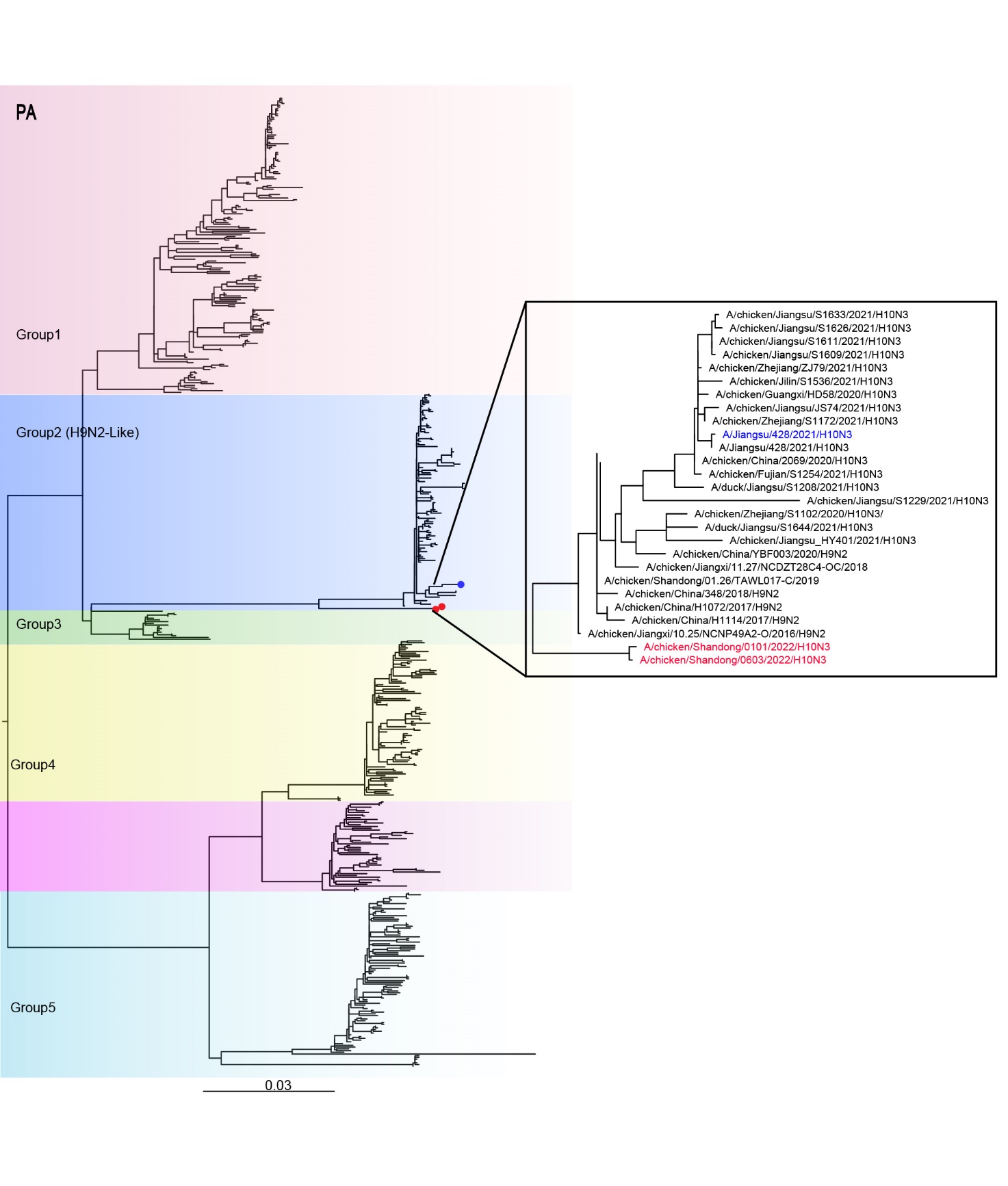
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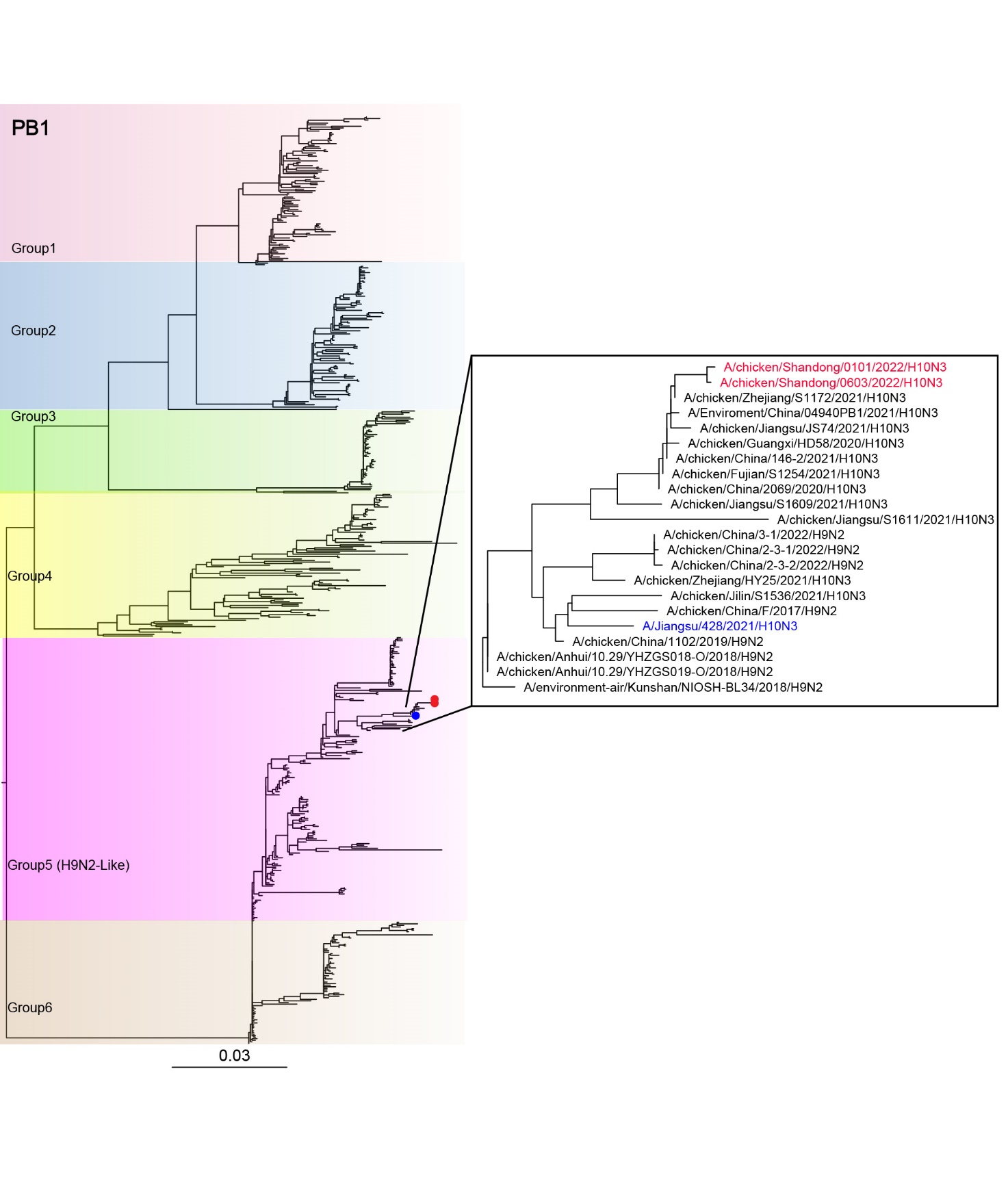
**Supplementary Figure S1.** Phylogenetic analysis of the internal genes (PB2, PB1, PA, NP, M, NS) of H10 subtype influenza viruses.

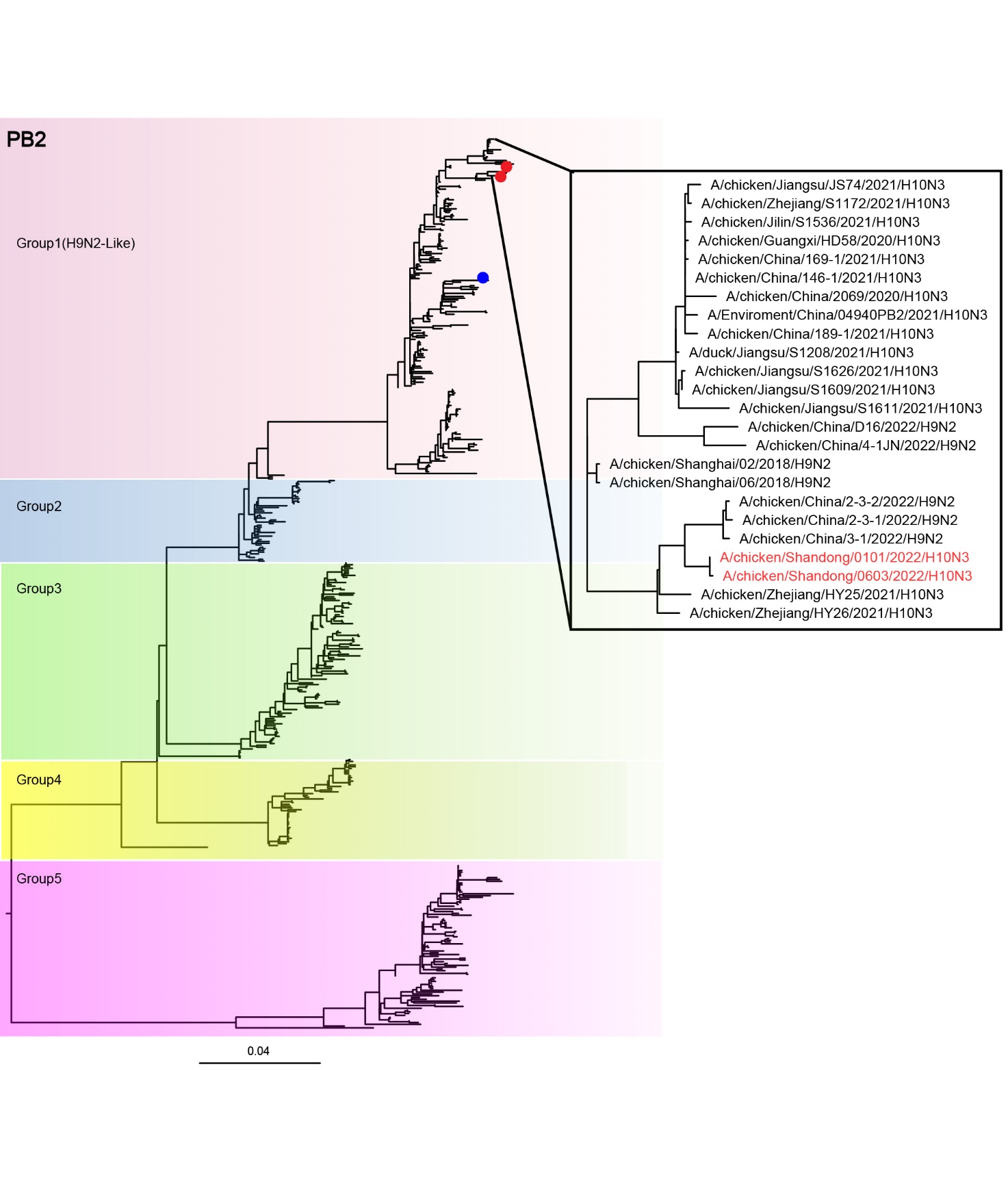


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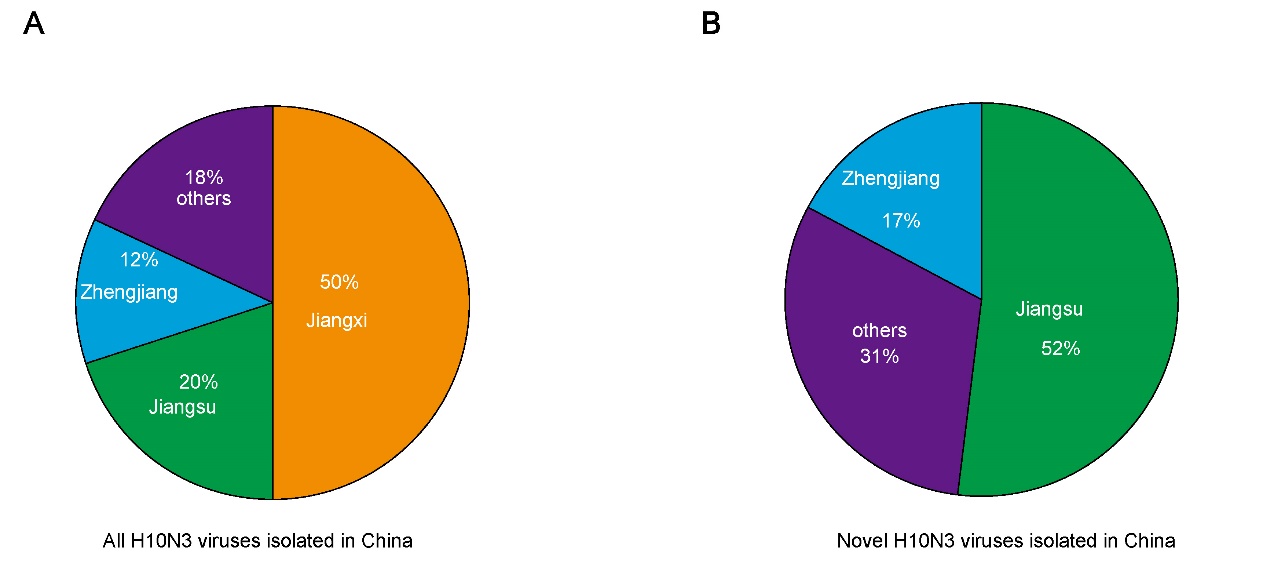
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**Supplementary Figure S2.** Provinces in China where the H10N3 virus is concentrated. **A** Percentage distribution of all H10N3 viruses isolated in China. **B** Percentage distribution of novel H10N3 viruses isolated in China.

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**Supplementary Table S1.** Supplementary references on adaptive mutations in this study.

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| Protein | Mutation | Function | Reference |
| HA | Q222R | Unknown |  |
| (H3 numbering) | G228S | Increased virus binding to α2–6 | Song et al., 2017 |
|  | Q226L | Increased virus binding to α2–6 | Qu et al., 2017 |
| Cleavage site | | Only one basic amino acid at the HA cleavage site, which is a characteristic of low-pathogenicity AIV in chicken | Zohari et al., 2014 |
| PB2 | E627K | Mammalian adaptive markers | Sediri et al., 2016 |
| D701N | Yu et al., 2019 |
| I292V | Suttie et al., 2019 |
| A588V | Suttie et al., 2019 |
| PB1 | I368V | Improved the polymerase activity and virulence in mammals | Guo et al., 2022 |
| S375N |
| PA | A343S | Increased the replication of viruses in mammalian cells and pathogenicity in mice | Yamaji et al., 2015 |
| K356R | Xu et al., 2016 |
| N409S | Yamayoshi et al., 2014 |
| M1 | N30D | Increased virulence in mice | Fan et al., 2009 |
| T215A |
| M2 | S31N | Resistance to adamantanes | Lan et al., 2010 |

PB, basic polymerase; PA, acidic polymerase; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix protein; NS, nonstructural protein.

0101, A/chicken/Shandong/0101/2022; 0603, A/chicken/Shandong/0603/2022; 0428, A/Jiangsu/0428/2021.

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