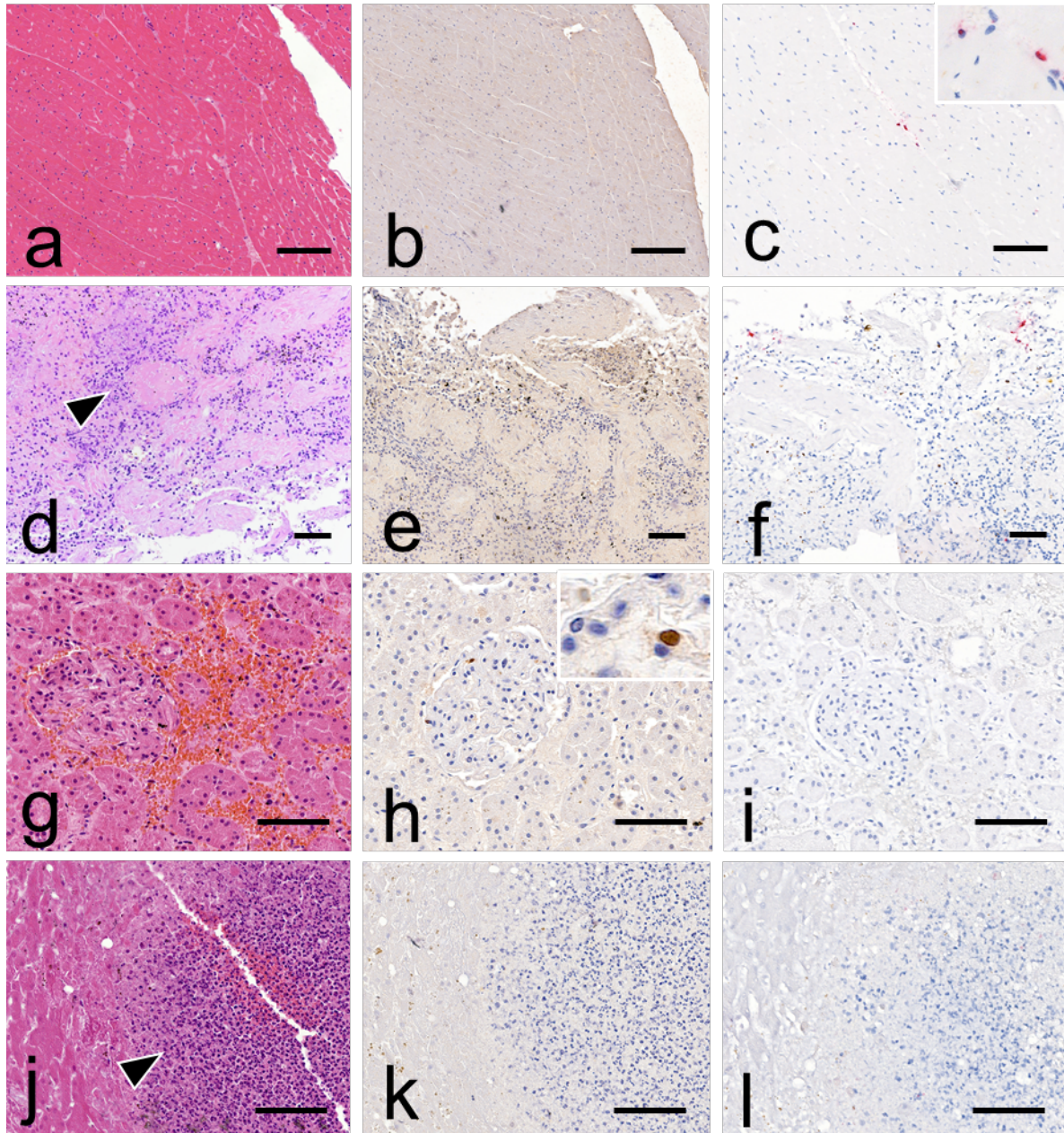


1 **Supplementary Figures 1 to 3**

2 Additional illustrations of histopathological findings, viral antigen and RNA distribution in
3 tissues from a Tibetan black bear and a black-headed gull naturally-infected with H5N1 clade
4 2.3.4.4b HPAIV.

5 **Supplementary figure 1.** Additional illustrations of histopathological findings, viral antigen and RNA distribution
6 in tissues from a Tibetan black bear naturally-infected with H5N1 clade 2.3.4.4b HPAIV.

7 a. Heart: the myocardium appears within normal limits (H&E stain). b. Heart: there is mild non-specific background staining
8 with no detection of viral antigen (IHC). c. Heart: sparse viral RNA detection is observed within the myocardial interstitium
9 (insert), M gene RNAscope ISH. d. Spleen: thrombosis is observed within the splenic parenchyma (arrowhead) (H&E stain). e.
10 Spleen: IHC reveals mild to moderate non-specific background staining with no significant detection of viral antigen. f. Spleen:
11 rare detection of viral RNA is observed within the red pulp (M gene RNAscope ISH). g. Kidney: there is congestion and
12 hemorrhages within the renal interstitium (H&E stain). h. Kidney: sparse viral antigen detection is observed within glomerular
13 tufts (IHC). i. Kidney: no viral RNA is detected (RNAscope ISH). j. Liver: the hepatic parenchyma is multifocally replaced by
14 nodular suppurative foci (arrowhead) (H&E stain). k. Liver: No viral antigen detection is observed (IHC). l. Liver: no viral RNA
15 is detected (RNAscope ISH). Scale bars: 50µm (d-l) 100 µm, 200 µm (a-c).



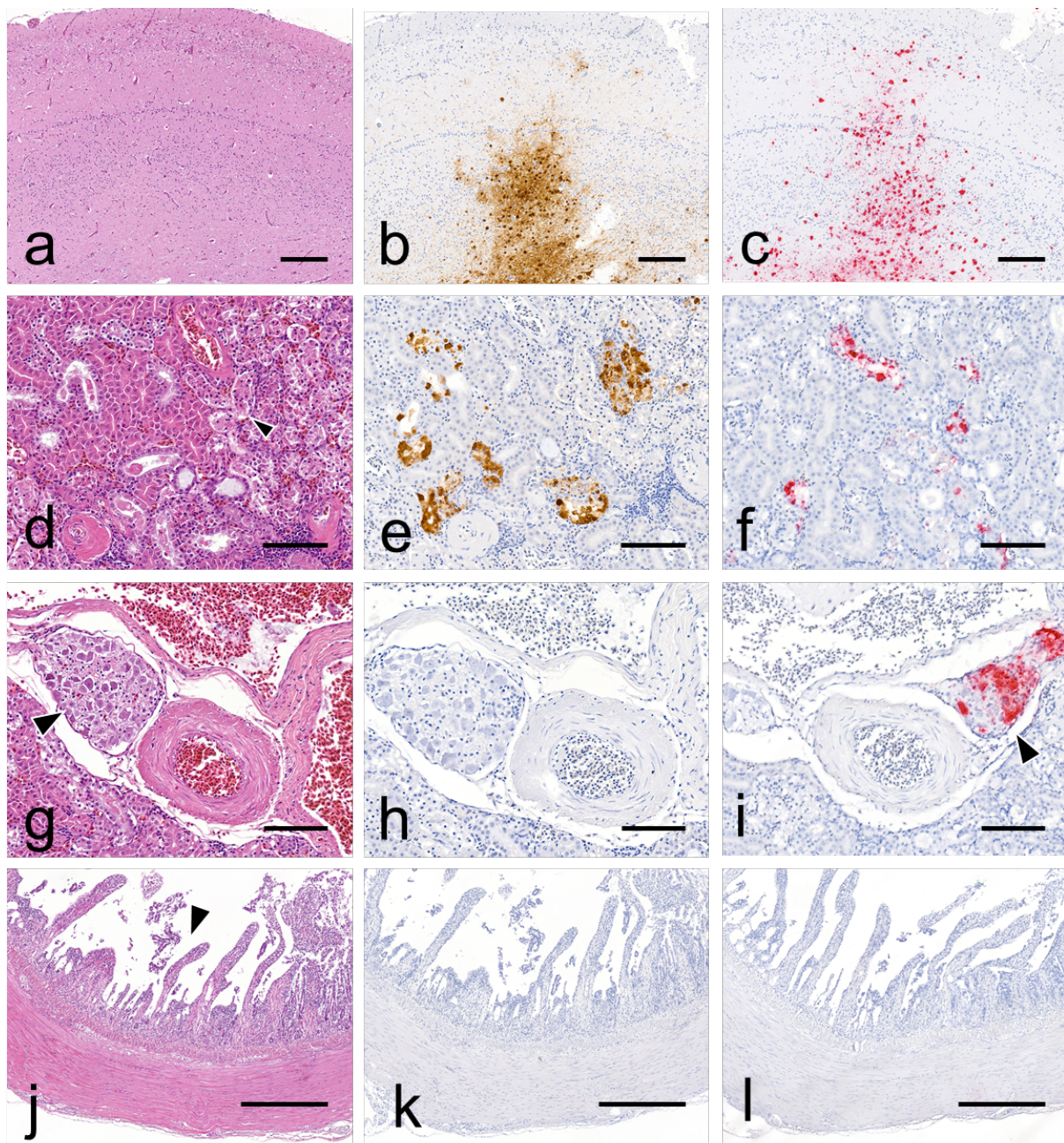
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19 **Supplementary figure 2.** Additional illustrations of histopathological findings, viral antigenic and RNA
20 distributions in tissues from a black-headed gull naturally infected with H5N1 clade 2.3.4.4b HPAIV.

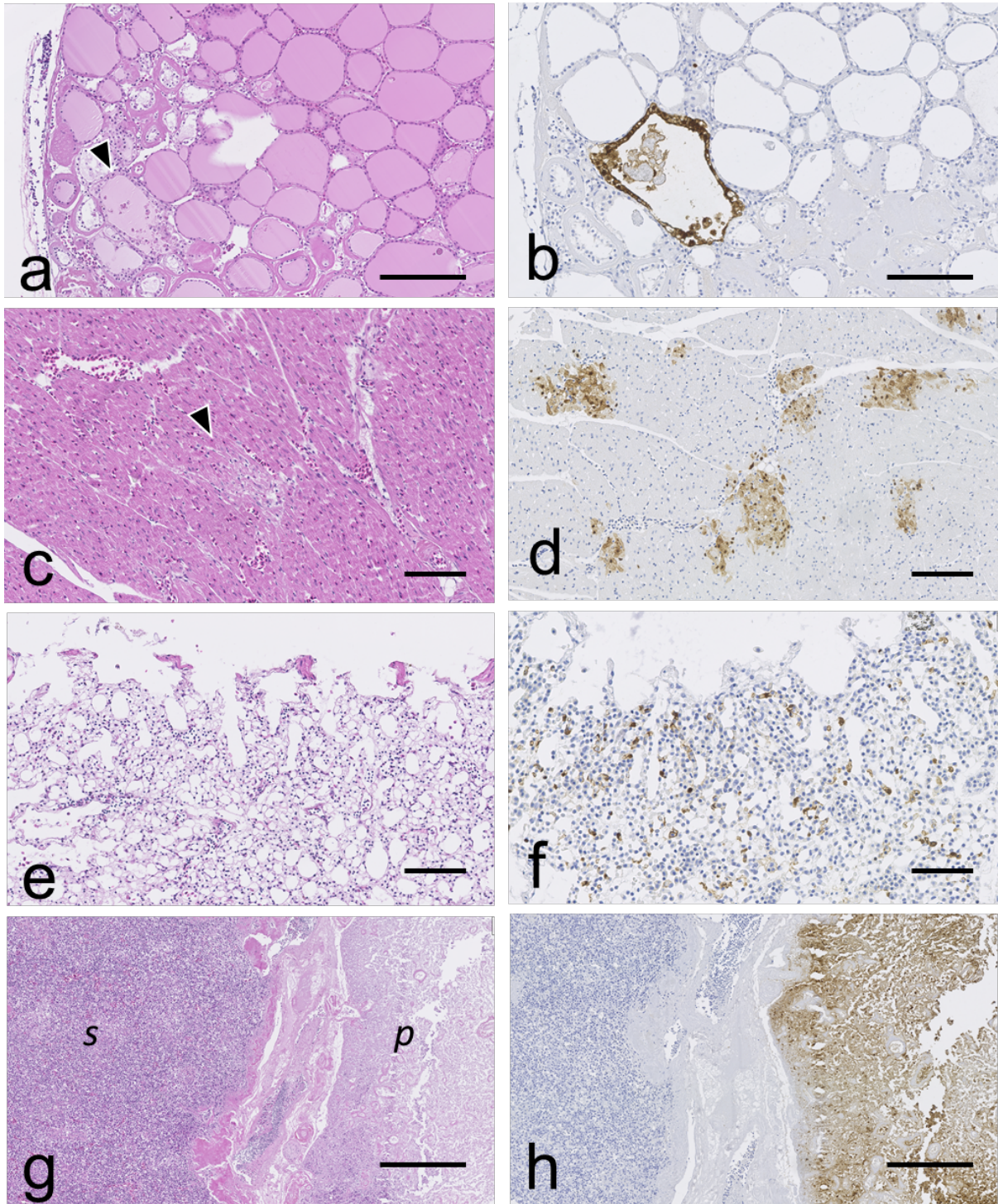
21 a. Optic Lobe (Central Nervous System, CNS): mild congestion is observed at a subgross view (H&E stain). b. Optic Lobe (CNS):
22 extensive viral antigen detection involving neurons, neuropil, and glial cells (IHC). c. Optic Lobe (CNS): extensive viral RNA
23 detection also involving neurons, glial cells and, to a lesser extent, neuropil (RNAscope ISH). d. Kidney: segmental multifocal
24 epithelial necrosis involving renal tubules (arrowhead) (H&E stain). e. Kidney: multifocal segmental positive viral antigen
25 detection within tubular nephrocytes (IHC). f. Kidney: multifocal segmental positive viral RNA detection within tubular
26 nephrocytes (RNAscope ISH). g. Prerenal Nervous Ganglion: within normal limits (arrowhead) (H&E). h. Prerenal Nervous
27 Ganglion: no viral antigen detection (IHC). i. Prerenal Nervous Ganglion: positive viral RNA detection within several neurons
28 (RNAscope ISH). j. Intestine: the mucosa exhibits epithelial sloughing (arrowhead), while the outer layers appear within
29 normal limits (H&E stain). k. Intestine: no viral antigen is observed (IHC). l. Intestine: no viral RNA is detected (RNAscope ISH).
30 Scale bars: 100 μ m (d-l), 200 μ m (a-c).



31

32 **Supplementary figure 3.** Additional illustrations of histopathological findings, viral antigen distribution in tissues
33 from a black-headed gull naturally-infected with H5N1 clade 2.3.4.4b HPAIV.

34 a. Thyroid Gland: degeneration and necrosis of thyroid follicles is observed (arrowhead) (H&E stain). b. Thyroid Gland: viral antigen detection involving both lining and sloughed epithelial cells of a single follicle (IHC).
35 viral antigen detection involving both lining and sloughed epithelial cells of a single follicle (IHC). c. Heart: mild focal myocardial
36 degeneration and necrosis (arrowhead) (H&E stain). d. Heart: multifocal viral antigen detection within cardiomyocytes (IHC).
37 e. Lung: pulmonary lobules appear within normal limits (H&E stain). f. Lung: multifocal viral antigen detection within the
38 capillary bed (IHC). g. Spleen and Pancreas (splenic Lobe): the splenic parenchyma (s) is within normal limits, while diffuse
39 pancreatic necrosis is observed (p) (H&E stain). h. Spleen and Pancreas (Splenic Lobe): no viral antigen is observed in the
40 spleen, while extensive positivity is present within the necrotic pancreatic parenchyma, presumably in exocrine acinar cells.
41 Scale bars: 100 μ m (a-f), 400 μ m (g, h).



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44

45 **Supplementary Figure 4**

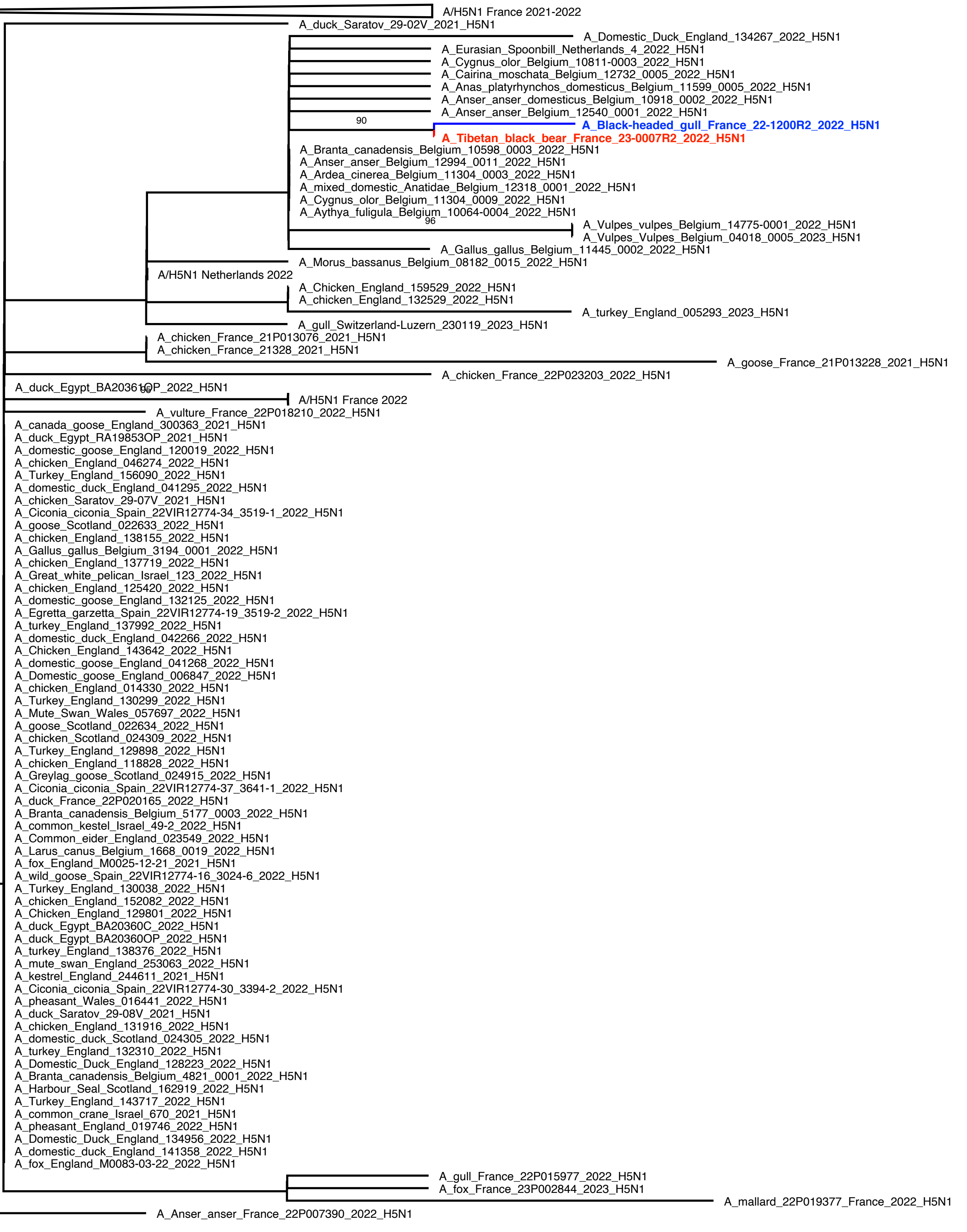
46 Maximum likelihood phylogenetic trees performed on viral segments other than HA. Bear and
47 gull-derived sequences are labelled in red and blue respectively. Bootstrap support values >
48 90 are shown at branches. Scale bar: number of nucleotide substitution per site.

PB2



0.002

M



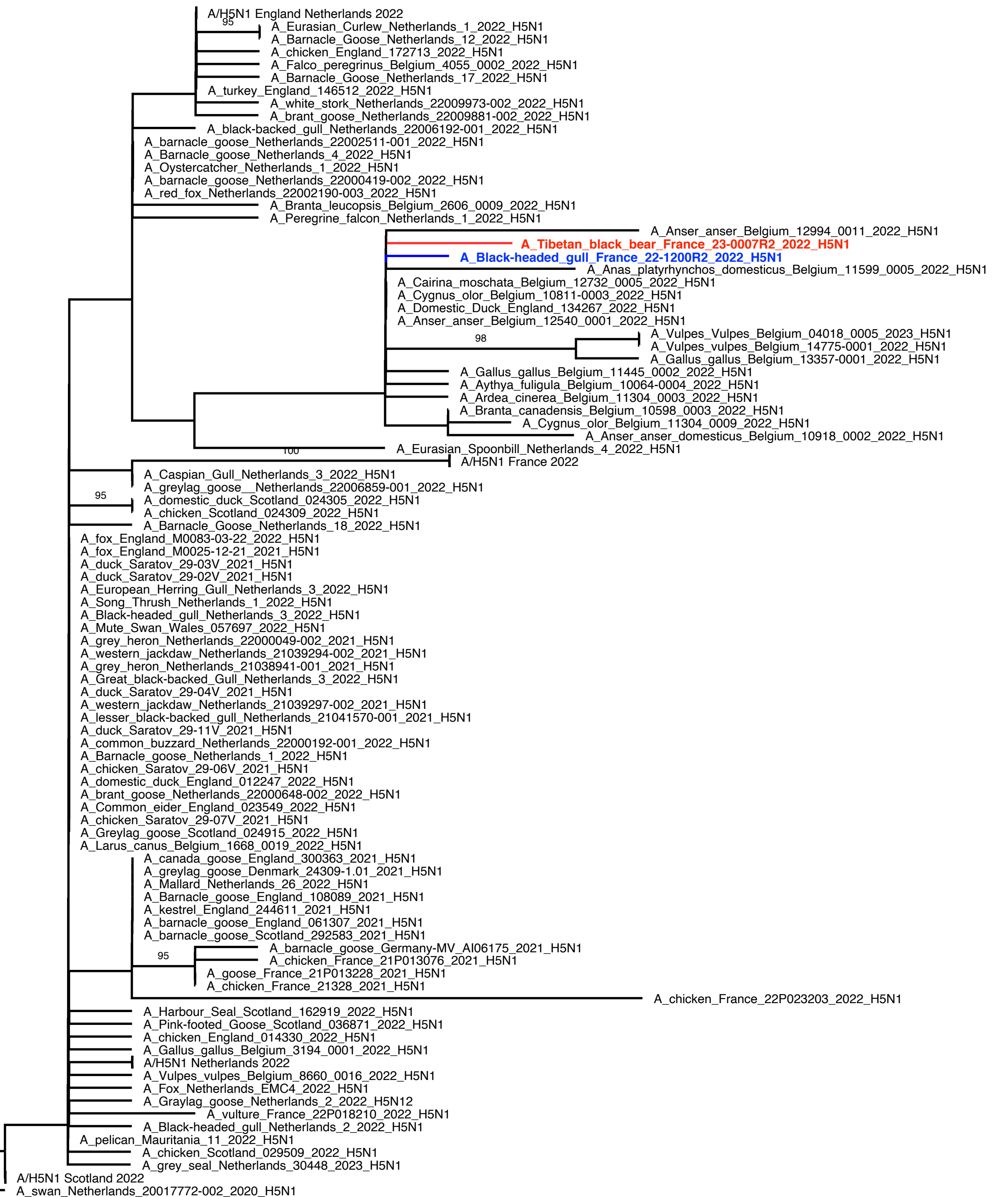
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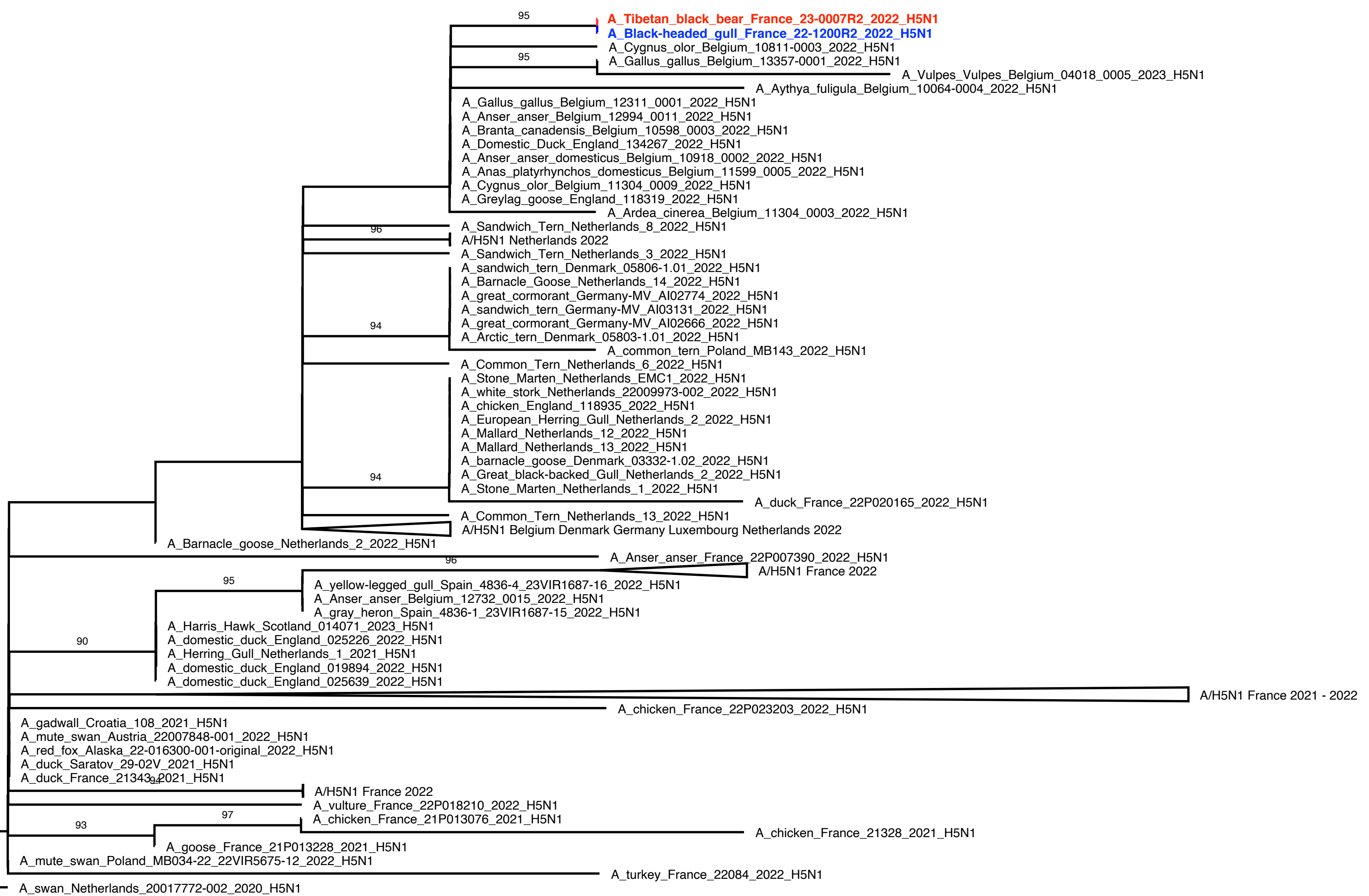


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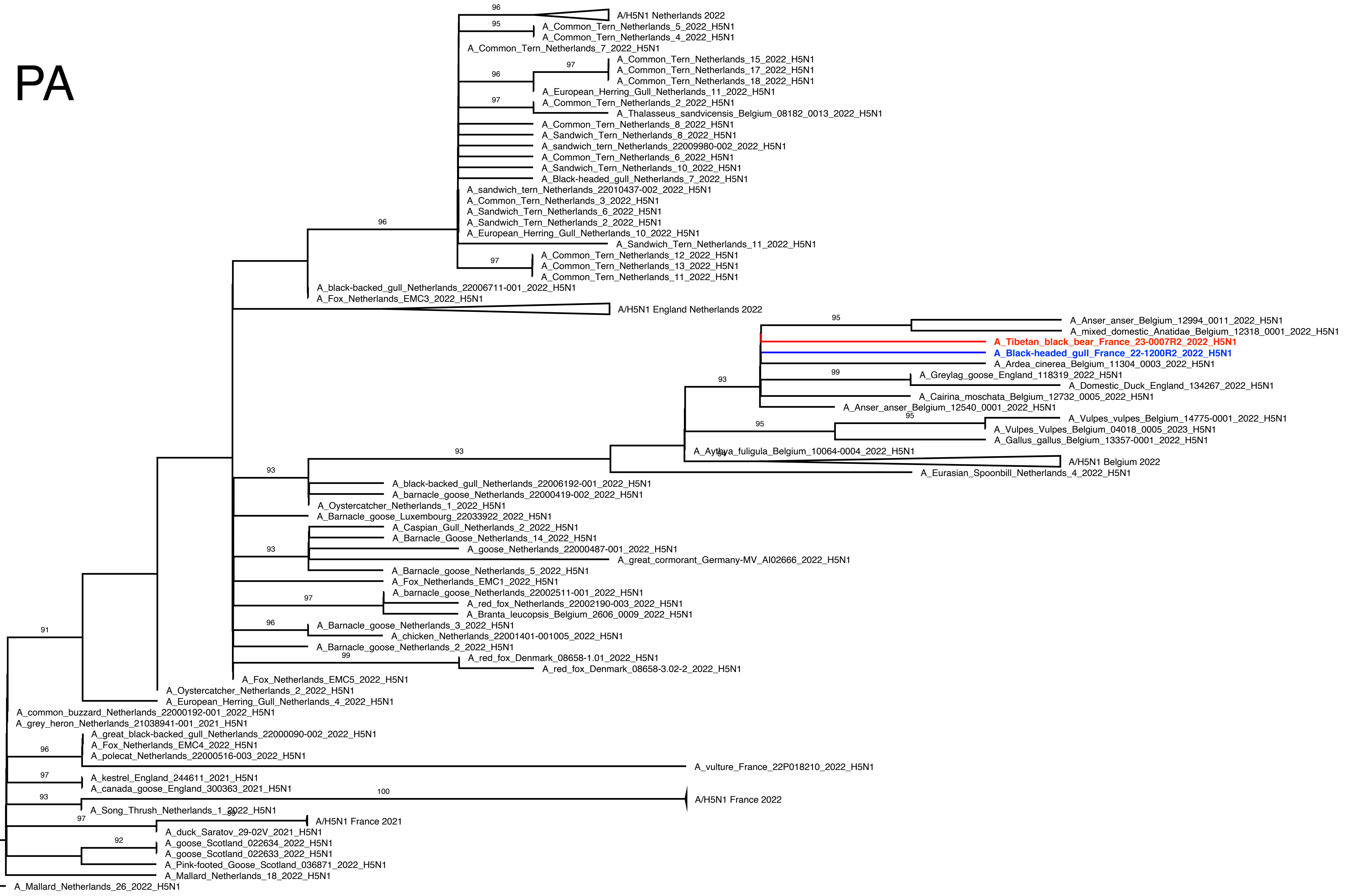


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0.002

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7.0E-4

PB1



0.002