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Authors: Seyed Ali Ghafouri, Mohammad Hossein Fallah, Seyed Farzad Talakesh, Hossein Hosseini, Zahra Ziafati, Mohammad Malekan, Leila Aghaeean, Arash Ghalyanchilangeroudi

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Full genome characterization of Iranian H5N8 highly pathogenic avian influenza virus from Hooded Crow (*Corvus cornix*), 2017: The first report

Seyed Ali Ghafouri[1], Mohammad Hossein Fallah[2], Seyed Farzad Talakesh[3], Hossein Hosseini[4], Zahra Ziafati[5], Mohammad Malekan[5], Leila Aghaeean[5], Arash Ghalyanchilangeroudi[5*]

1Iranian Veterinary Organization, Tehran, Iran.

2Department of Poultry Diseases, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.

3Iranian Egg Farmers' Federation, Secretory-General, Tehran, Iran.

4Department of Clinical Sciences, Faculty of Veterinary Medicine, Islamic Azad University, Karaj Branch, Karaj, Iran

5Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Corresponding Author:

Arash Ghalyanchilangeroudi
Department of Microbiology and Immunology,
Faculty of Veterinary Medicine, University of Tehran,
Tehran, Iran.
Email: arashghalyanchi@gmail.com
Tel: 00982161117154

Highlights:

- Identification of H5N8 highly Pathogen Avian Influenza Virus from Hooded crow (Corvus cornix) in a national park located at Esfehan province in Iran.
- Based on HA sequencing results, it belongs to 2.3.4.4 clade, and the cleavage site is (PLREKRRKR/G).
- Complete genome characterization of this virus revealed probable reassortment of the virus with East-Asian low-pathogenic influenza viruses.

- A mutation at antibody binding site of hemagglutinin A201E of Aghakhan revealed the probable initialization for antigenic drift of this virus
- phenotypic markers related to the increased potential for transmission and pathogenicity to mammals were observed

Abstract:

During 2014-2017 Clade 2.3.4.4 H5N8 highly pathogenic avian influenza viruses (HPAIVs) have spread worldwide. In 2016, an epidemic of HPAIV H5N8 in Iran caused mass deaths among wild birds, and several commercial poultry farms and captive bird holdings were affected and continue to experience problems. Several outbreaks were reported in 2017. One of them is related to Hooded crow (*Corvus cornix*) in a national park in Esfahan province in 2017. Whole genome sequencing and characterization have been done on the detected H5N8 sample. Based on HA sequencing results, it belongs to 2.3.4.4 clade, and the cleavage site is (PLREKRRKR/G). Phylogenetic analysis of the HA gene showed that the Iran 2017 H5N8 virus clustered within subgroup Russia 2016 2.3.4.4 b of group B in H5 clade 2.3.4.4 HPAIV.

On the other hand, the NA gene of the virus is placed in group C of Eurasian lineage. Complete genome characterization of this virus revealed probable reassortment of the virus with East-Asian low-pathogenic influenza viruses. Furthermore, the virus possessed some phenotypic markers related to the increased potential for transmission and pathogenicity to mammals at internal segments. This study is the first full genome characterization H5N8 HPAIV in Iran. The data complete the puzzle of molecular epidemiology of H5N8 HPAIV in Iran and the region. Our study provides evidence for fast and continuing reassortment of H5 clade 2.3.4.4 viruses, that might lead to changes in virus structural and functional characteristics such as the route and method of transmission of the virus and virus infective, pathogenic and zoonotic potential.

Keywords: Avian Influenza, Iran, H5N8, Phylogenetic study, Crow, Full genome, Characterization

Introduction:

Avian influenza viruses (AIVs) are not only important pathogens for birds but also they are important in public health due to cause sporadic and non-sustained infections in human populations. The H5 viruses have evolved into ten distinct phylogenetic clades (clades 0–9) by the HA gene of isolates from 1996-2006 in China [1]. H5N1 highly pathogenic avian influenza viruses (HPAIVs) of the A/Goose/Guangdong/1/1996 (Gs/Gd) lineage have become panzootic in domestic birds in Eurasia and Africa, since they detect for the first time) [2]. HPAI A/duck/Jiangsu/k1203/2010 H5N8 virus of the Asian H5N1 lineage (HA gene belonging to clade 2.3.4) was initially isolated from mallard ducks at a live-bird market in eastern China in 2010 [3, 4].

In 2013, live poultry markets in eastern China was the first place for isolation of novel reassortant H5N8 viruses and then the virus detected in poultry and wild birds in the Republic of Korea and Japan [5].

Two distinct genetic groups of HPAI H5N8 were identified in phylogenetic analysis in the Republic of Korea and each group represent by a characterized virus: group A (A/broiler duck/Korea/Buan2/2014-like) and group B (A/breeder duck/Korea/Gochang1/2014-like). In late 2014, HPAI H5N8 viruses were reintroduced into South Korea and Japan and they discovered in Europe and North America [2]. Some incidents of HPAI are reported in Iran: H5N1 and H5N8. The H5N1 subtype was first detected and confirmed in wild swan corpse on routine surveillance in Iran on February 13, 2006 [6]. Also, In November 2016, HPAI H5N8 was detected in a commercial egg farms in the province of Tehran [7]. Genetic and phylogenetic analysis of the HA gene demonstrated that the Iranian HPAI H5N1 and H5N8 viruses belong to the HPAI H5 virus clades (2.2.1, 2.2.2 and 2.3.2.1c) and 2.3.4.4, respectively [6, 7].

Transcontinental migration of wild birds has role in spread of HPAI H5 viruses in Asia, Europe, and North America which lead to zoonosis concern in the world [1]. Furthermore, the poultry trade is believed to have caused the spread of the virus, and it has affected poultry production

and public health mainly in Asian countries [8]. In our study, new H5N8 viruses were isolated from Hooded crow (*Corvus cornix*) in a national park in the Iranian province of Esfahan in 2017. We sequenced the entire viral genome, performed the phylogenetic analysis, and determined the molecular characteristics of the virus.

Material and Methods:

Sample History:

The province of Isfahan is one of the most important poultry breeding sites in the country. In the case of H5N8 in 2017, this province was also the subject of disease and four outbreaks were identified there [9]. One of the outbreaks was in the National Park in Falavarjan where ten dead Hooded crows Corvidae suspected of highly pathogenic Avian Influenza were destroyed. Trachea, cecal tonsils, lungs, brain, and spleen were collected according to the standard method from mortalities of the above-mentioned birds and, under strike biosecurity protocols, were immediately transferred to central laboratory of the Department of Clinical Sciences at the University of Tehran and were stored in -70 °Cfor further examination. All of the samples in this outbreak were analyzed, and all bases on partial HA gene sequences were located in the same group with 98.90%-100% similarity.

RNA Extraction:

The viral RNA extracted from sample tissues via High Pure viral nucleic acid kit and according to the manufacturer's instruction (Roche, Mannheim, Germany).

RT-PCR for genes amplifications:

Reverse transcription was done by utilization of oligonucleotide influenza universal primer uni12: 5-AGCAAAAGCAGG-3 with RevertAid First Strand cDNA Synthesis Kit (Fermentas, Canada).

Full-length amplification of all eight segments of the viral genome was performed by usage of Hoffmann et al. universal primers [10]. For each gene amplification a 50 µl total volume of master mix contained 5 µl PCR buffer 10X, 20 pmol of each forward and reversed primer (2 µl), 2 µl dNTP 10 mM, 4 µl MgCl2 50 mM, 0.5 µl Pfu DNA polymerase (Fermentase, Canada) and 2 µl cDNA

samples. The thermo-cycler protocol was: denaturation at 94°Cfor the 4-minutes period and then 30 cycles of 94°Cfor 20 seconds and 58°Cfor 30 seconds and a final extension at 72 for 7 minutes. Ten µl of the PCR products were loaded by EvaGreen (Jena Bioscience, Germany) loading buffer on 1% agarose gel near 100bp Marker (Sianclon, Iran) in 1x TBE buffer and were electrophoresed [11].

Sequencing and Bioinformatics analysis:

The AccuPrep® PCR purification Kit (Bioneer Co., Korea) was used for purification of the PCR products. Sequencing was performed using ABI 3100 Genetic Analyzer (Applied Biosystems, USA) with the primers (Both directions) (Bioneer Co., Korea). Chromatograms were evaluated by utilization of CromasPro software (Version 1.5). After sequencing, all sequences from a given sample were combined and used to NCBI BLAST for confirmation and construct alignments with H5N8 sequences retrieved from NCBI and GISAID's EpiFluTM Database (http://platform.gisaid.org/epi3/frontend). The sequences of each of the eight genetic segments were aligned by Clustal W method. 1,000 bootstrap replicates was used for phylogenetic trees construction by the Neighbor-joining method using MEGA 7 software [12]. The phylogeny test options used to construct the trees were, complete deletion of gaps/missing data, and nearest neighbour interchange for the heuristic method. The Kimura 2-parameter model was used. The sequences of the eight segments of the H5N8 virus have been deposited in GenBank, and the accession numbers are MK168596-MK168603. The single-letter codes were used to express all amino acids. Identification and comparison of N-glycosylation sites of NA protein sequences were performed by an online server ScanProsite (http://prosite.expasy.org/scanprosite).

Results:

Phylogenetic results:

Phylogenetic analysis of the HA gene showed that the Aghakhan virus clustered within the subgroup Russia 2016 2.3.4.4 b of group B in H5 clade 2.3.4.4 Highly Pathogenic Avian Influenza Virus (HPAIV). On the other hand, the NA gene of Aghakhan placed in group C of Eurasian lineage

which included recent viruses isolated from Korea, Cameron and Nur-Lake outbreaks (Fig. 3). However, Complete genome characterization of this virus revealed probable reassortment of the virus with East-Asian low-pathogenic influenza viruses (LPAIVs) (Fig. 1). Notably, the PB2, PB1, M, and NS genes of the Aghakhan virus clustered with isolates identified in Korea, Western Siberia (Uvs-Nuur Lake), China (Qinghai Lake), Europe, and India in 2016-2017. Furthermore, the PB1 segment of Aghakhan virus came from A/wild duck/Korea/SH5-26/2008(H4N6). Also, the PA gene of the Aghakhan virus was phylogenetically distinct from those of H5N8 viruses from Uvs-Nuur Lake and Qinghai Lake but clustered beside an Indian isolate (A/painted stork/India/10CA03/2016, India 10CA03), an Italian isolate (A/turkey/Italy/17VIR538-1/2017), and a Russian isolate (A/gadwall/Chany/97/2016, Chany 97). The NP gene due to reassortment phenomenon was phylogenetically distinct from H5N8 viruses identified at Qinghai Lake, Uvs-Nuur Lake, India (India 10CA03), Russia (Chany 97), and Europe.

Amino Acid Analysis:

We considered the Aghakhan virus to be an HPAIV by the deduced amino acid sequence at the HA proteolytic cleavage site (PLREKRRKR/G). In the HA, the receptor-binding sites maintained Q226 and G228 (H3numbering), which is suggestive of preferential binding to the sialic acid-2,3-NeuAcGal, as is typical for avian influenza viruses. A mutation at antibody binding site of hemagglutinin A201E of Aghakhan revealed the probable initialization for antigenic drift of this virus (Table 1). However, such mutation was not present at H5N1 isolated at 2011 in Iran. The established markers of NA inhibitor resistance (E119G, Q136K, D151A, D198G, I222R, H274Y, R292K, N294S, and R371K; N2 numbering) and amantadine resistance (V27A and S31N, M2 protein) were not found [8].

The HA of Aghakhan virus showed the highest similarity with Israeli, Korea, and Cameroon isolates. Also, the NA revealed more 98% similarity with Cameron and Korea isolates. Regarding internal genes, the highest similarity was observed between Aghakhan and Korean, Cameron, Russian, Italian and Egypt isolates (Table 2).

Genetic Analysis of internal genes:

Furthermore, phenotypic markers related to the increased potential for transmission and pathogenicity to mammals such as markers in PA gene I78, S228, D272 and V379, in PB1 gene L598 and K737, in NS1 gene S42 and in PB2 gene V89, D309, K339, G477, V495, E627, T676 and D701were observed. The M1 gene of Aghakhan revealed Aspartic acid and Alanine in positions 30 and 215 receptively.

Aghakhan illustrated five potential N-linked Glycosylation sites at amino acids 54, 67, 84, 144 and 293 in the NA proteins.

Discussion:

AIV surveillance in domestic poultry and wild birds is critical to our understanding of the persistence, transmission, and evolution of AI viruses. It is believed that wild birds have played an important role in dissemination of Goose/Guangdong/1/1996 (Gs/GD) lineage HPAIV, as seen in the spread of clade 2.2 H5N1 HPAIV from Qinghai Lake and circumpolar breeding areas to Europe and East Asia in 2005-2006 and clade 2.3.4.4 H5N8 HPAIV from Siberia to Europe, Asia, and North America in 2014. Since 2014, outbreaks of clade 2.3.4.4H5 HPAIV have been reported in various geographic regions, and they have evolved into multiple genotypes. Enhanced surveillance and comparative genetic analysis will help to monitor the further evolution and dissemination of clade 2.3.4.4 HPAIVs.

In this study, phylogenetic analysis reveals that Aghakhan virus is clustered beside other H5N8 Highly Pathogenic Avian Influenza isolates from Korea, Egypt and Cameron in Russian 2016 subgroup b of group B in clade 2.3.4.4 of H5 viruses. As Iran situated in the West Asian-East African and Black Sea-Mediterranean flyways, the hypothesis that the H5N8 virus was introduced via migratory birds to local poultry industry is very strong (Fig 2). This pattern of this spread is quite similar to outbreaks of H5N1 between 2003-2005. Also in H5N1 outbreak China, Korea, Mongolia, Russia, Iran, Egypt, and Cameron were involved [13].

Stallknecht and Shane isolated 2317 influenza viruses out of 21,318 samples from all bird species. The highest prevalence was recorded from Anseriformes, then Passeriformes and Charadriiformes, respectively [14]. Tanimura et al. demonstrated that the crows were susceptible

to infection with the H5N1 virus and the virus infection led to degenerative and necrotic lesions in the lung, heart, central and peripheral nervous system, alimentary tract, pancreas, kidney, adrenal gland, and testis [15]. Desvaux et al. isolated HPAI H5N1 from Corvus macrobenthos (large-billed crow) in an aviary in Cambodia [16]. Moreover, Majidzadeh et al. detected avian influenza in different species including duck, swan, parrot, crow, pigeons, and chicken at the Tehran Bird park [17]. Although migratory birds are known as the primary introduction source of HPAI in an area, secondary means the spread of infection should not be neglected. For Example, flying insects could become contaminated with infected faeces and transfer contamination from bird to bird. Also for longer distances inside a region trucks and other vehicles can affect the spread of the virus considerably [18]. A study revealed that the virus from *Calliphora nigribarbis* (Blow Fly), namely A/blowfly/Kyoto/93/2004 (H5N1), was the same strain of the virus from infected chickens (A/chicken/Kyoto/3/2004, (H5N1)) and crows (A/crow/Kyoto/53/2004, (H5N1)) in Kyoto. The blowflies actively ingested the virus through droppings and secretions of infected birds. Chickens and many wild birds eat flies, even when they are flying [19].

In some cases, such as that of crows in Japan, the wild birds were believed to have been infected by diseased poultry or infected captive birds rather than having been the source of the virus for poultry [20]. These local wild birds might have been infected as spillover hosts from the disposal of dead poultry [21]. Field surveys show that these birds are likely to be infected due to carcasses of farmed turkeys infected in the Najaf Abad (near this area) abandoned by farm owners. The identification of the virus in the lost crows has elevated the importance of the role of these birds in the transmission of viruses, given their free flow and their displacement between different regions and in particular industrial farms.

Epidemiological studies illustrated the existence of a migration route for birds carrying H5N8 HPAIV from East Asia to Russia [8]. In a study using clade 2.3.4.4 H5N8 HPAI viruses showed that they replicated systemically and were lethal in chickens but appeared to be attenuated, although efficiently transmitted, in ducks. A range of outcomes of infection from no clinical signs to severe disease was observed in ducks' internal nasal inoculated with H5N8 viruses, and the mortality

rates varied from 0 to 20% [22]. This evidence can confirm the role of migratory birds in the transmission of HPAI H5N8 across vast distances.

As the first step in influenza infection, binding of the HA to human cell-surface receptors with appropriate affinity and specificity is a crucial requirement for viral infection and spread as well as for assessing a human pandemic potential [23]. α-2,6-linked sialic acid receptors on cells on the cells of the upper respiratory tract are preferable receptors for human influenza viruses. Although there are some α -2,3-linked sialic acid receptors in the alveolar cells of the human respiratory system, avian-adapted influenza viruses bind preferentially to the α-2,3-linked sialic acid receptors abundant in the gastrointestinal tract of birds [24]. The single amino acid substitutions A138S, G186V, Q226L, Q196H, Q196R, S227N, and N224K and the combination of amino acid substitutions Q226L and G228S in the HA protein of the influenza A virus has been reported to increase the affinity of HA for the human receptor, sialic acid linked to galactose by alpha 2,6 linkages [8]. As Aghakhan virus did not reveal these substitutions, it could be concluded that this virus HA preferred alpha 2,3 linkage receptors to bind (Table 1). Furthermore, Receptor-binding sites (Gln226 and Gly228) of the H5N8 subtype viruses suggests that these viruses would preferentially bind to avian-like receptors [5], as it revealed in recent Iran H5N8 isolate. The mutation A201E was found in the A/chicken/Egypt/Q1769B/2010(H5N1) strain and also Russian H5N8 isolates of 2014, detected in Egypt in May 2010 [25].

In the phylogenetic tree, the NA gene of Aghakhan was clustered beside all other H5N8 isolates of the year 2016 and 2017 in a separate group (group C) in the Eurasian lineage. This finding indicates the H5N8 viruses are continuously evolving. Five potential N-glycosylation sites of the Aghakhan NA protein were the same as H5N8 isolates of 2014 China isolates [4]. Phylogenetic trees of internal genes illustrated that the PA, NS, PB1, PB2, and M of Aghakhan are genetically more closely related to Korean H5N8 isolates of 2016 and 2017. The NP gene of Aghakhan showed higher similarity with European H5N8 isolates of the recent years. However, originally this segment belonged to H10N7 LPAI circulating in China in 2009 (Fig. 1). All analysed H5N8, and H5N5 viruses 2016-2017 from Italy revealed reassortment in the NP segment.

A/widgeon/Italy/16VIR9616-3/16 (H5N5) and A/gadwall/Italy/17VIR133-7/17 (H5N5) took their NP segment from A/Chicken/Scotland/532/2016 LPAI H5N1, and A/turkey/Italy17VIR538-1/17 possessed NP segment from A/Pigeon/Egypt/S10409A/2014 H9N2 [26]. The putative role of NP as a determinant of host range has led to its use as a model for the long-term host-specific evolution of influenza viruses. It is believed that geographic patterns of evolution are evident in avian virus NP genes; North American, Australian, and Old World isolates from separate sublineages [27]. All genome segments of the novel H5N8 clade 2.3.4.4 from group B strains from Germany in 2016 revealed major similarities with the Russia–Mongolia border isolates (e.g., A/great crested grebe/Uvs-Nuur Lake/341/2016) and The NP segment sequences of Russia–Mongolia border isolates are similar to those of Low Asian pathogen avian influenza viruses[28]. Based on the phylogenetic study, the Aghakhan illustrated more similarity to the recently circulating strains causing outbreaks in Asia, Africa, and Europe. The PB1 of Aghakhan illustrated reassortment A/wild duck/Korea/SH5-26/2008(H4N6) LPAI. In the case of PB1 genes, virus-specific constraints on the viral polymerases may prevent any significant host-specific divergent evolution [27]. It is suggested that the PB1 could have a specific role in increased virulence of virus, replication efficiency and antivirus response in mice [29]. Aghakhan possessed Mutations at amino acids numbered N30D and T215A in the M1 gene which is effective on increasing virulence of H5N1 in mice [30]. Studies showed Efficient replication at lower temperatures is considered to be one factor that may restrict viral host tropism. Human influenza viruses replicate efficiently in the human upper respiratory tract in which the temperature ranges from 33 to 34 °C, whereas the avian intestinal tract with approximate temperature 39–41 °C is preferable for sufficient replication of avian influenza viruses. Mutations of K737E in PB1, S63F in PB1-F2, and I78V, S225R, D272G, and V379M in PA and 5 amino acids in PB2 might contribute to efficient replication at 33 °C [2].

On the other hand, Amino acid changes L89V, G309D, T339K, R477G, I495V, and K627E in PB2 were noted in the H5N1 2014 isolate. These substitutions in conjunction with changes in the M1 and HA proteins have been described to enhance polymerase activity and virulence in mice [30].

It was suggested that the presence of Glu at position 627 of the PB2 gene could contribute to inefficient replication at lower temperatures. It was proposed that viruses with avian or avian-like surface glycoproteins reveal a reduced capacity to establish productive infection at the temperature of the human proximal airways [31]. Also, The PB1 protein showed the P598L mutation reported to enhance polymerase activity in mammalian cells and mice [30]. Except for PA, Aghakhan virus showed all mentioned mutations, and it could be concluded that the virus has the potential to replicate in mammalian cells and some mutations in HA gene will increase the ability of the Aghakhan virus to invade mammalian hosts.

Aghakhan possesses S42 in NS1 Protein. It was demonstrated that the amino acid S42 of NS1 is critical for the H5N1 influenza virus to antagonize host cell interferon induction and for the NS1 protein to prevent the double-stranded RNA-mediated activation of the nuclear factor-κB pathway and the interferon regulatory factor -3 pathway. Thus, the NS1 protein is critical for the pathogenicity of H5N1 influenza viruses in mammalian hosts and serine in amino acid position 42 of NS1 plays a key role in imbibing the antiviral immune activity of the host cell [32].

In conclusion, we must pay attention to a pathogenicity study on H5N8 that revealed the rapid excretion of the virus coupled with the limited morbidity it produces could present a significant challenge to virus monitoring efforts in domestic farms. If poultry owners rely mainly on mortality (due to exacerbated disease) or reduced egg production as visible criteria for detection, the virus could spread long before the infection is suspected or confirmed. Hence, apart from migratory wild birds, the movement of asymptomatic ducks as well as local wild birds may have also contributed to the silent spread of the virus that gave rise to the recent unprecedented and widespread poultry outbreaks [33]. As Iran is located in the migratory bird's flyways and intensive domestic and industrial poultry farms are centered in different parts of Iran, the national surveillance system must be cognizant of new HPAI virus introduction and transmission and the current virus evolution in industrial poultry farms, migratory birds, backyard poultries and local wild birds. Because these types of viruses can reassert with other Influenza viruses circulating in the area especially human influenza viruses and increase their adaptation to

mammalian hosts. On the other hand, although the 2017 isolate of H5N8 from Iran demonstrated lake of mutations responsible for increasing affinity of HA protein to alpha 2,6 receptors, the virus harboured other mutations in internal genes with a critical role on increasing virulence of the virus in mammalian hosts. Thus, intensive studies needed for investigation of evidence of H5N8 HPAI evolutions.

"Compliance with Ethical Standards"

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Fig 1. Schematic pattern of A/Crow/Aghakhan/2017 Highly Pathogenic Avian Influenza H5N8 of Clade 2.3.4.4 Bb genotype isolated in this study. Reassortant Aghakhan compromise PB1 and NP segments from East Asian Low Pathogenic Avian Influenza. (PB1: Polymerase Basic 1; PB2: Polymerase Basic 2; PA: Polymerase Acidic; HA: Hemagglutinin; NP: Nucleoprotein; NA: Neuraminidase; M: Matrix; NS: Non-structural)

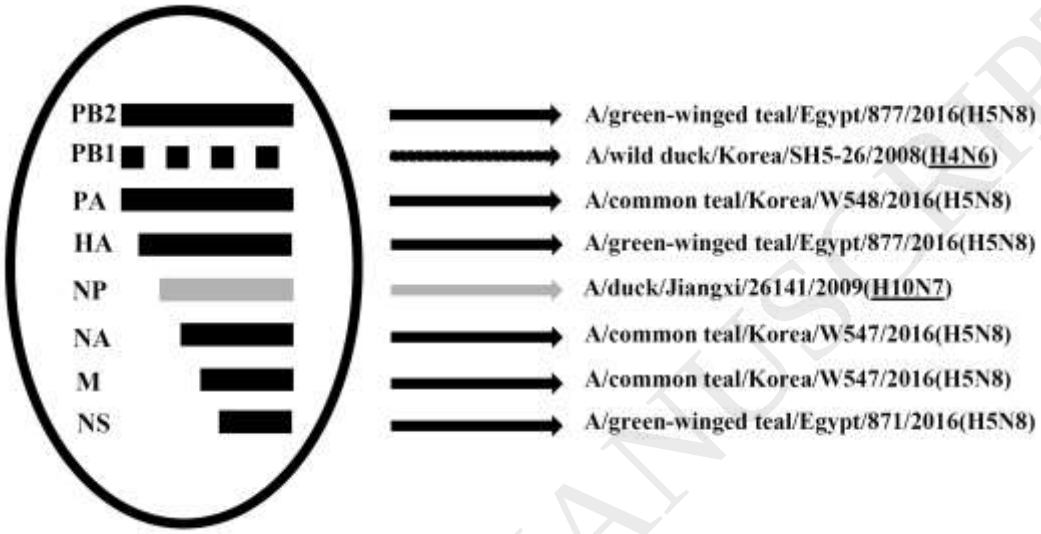


Fig 2. Based on phylogenic analysis of HA gene, all H5N8 clustered beside Aghakhan HPAI H5N8 virus were isolated in rather same time and could emerge from the same ancestor. Based on locations, Central Asian flyway, West Asian-East African flyway, and Black Sea-Mediterranean flyway could have an effective role in the spread of the HPAI H5N8 viruses.

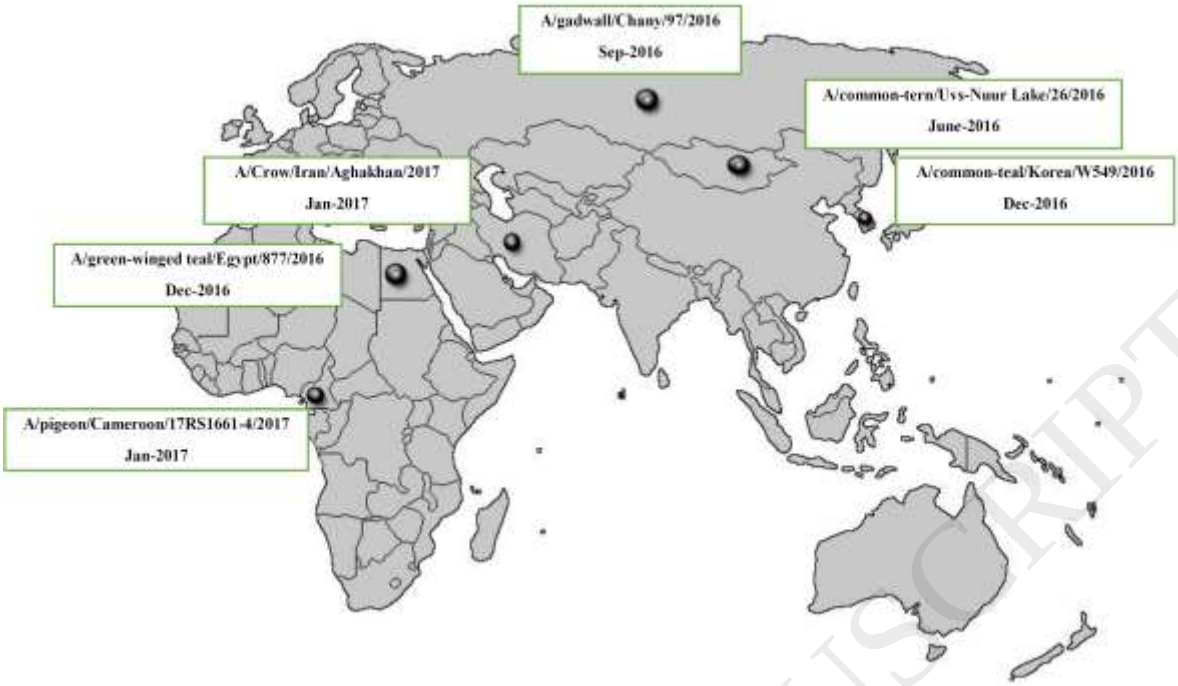
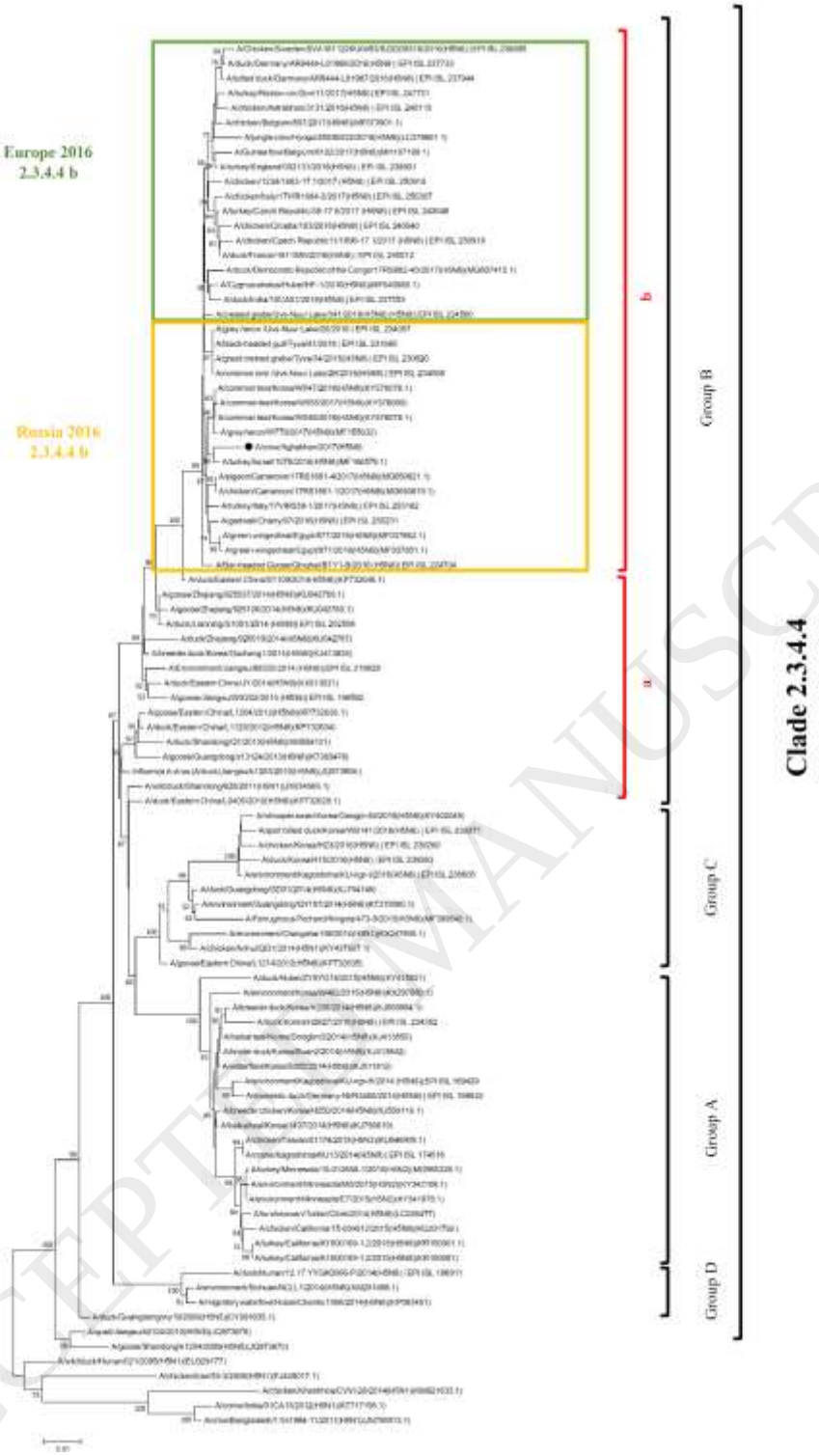
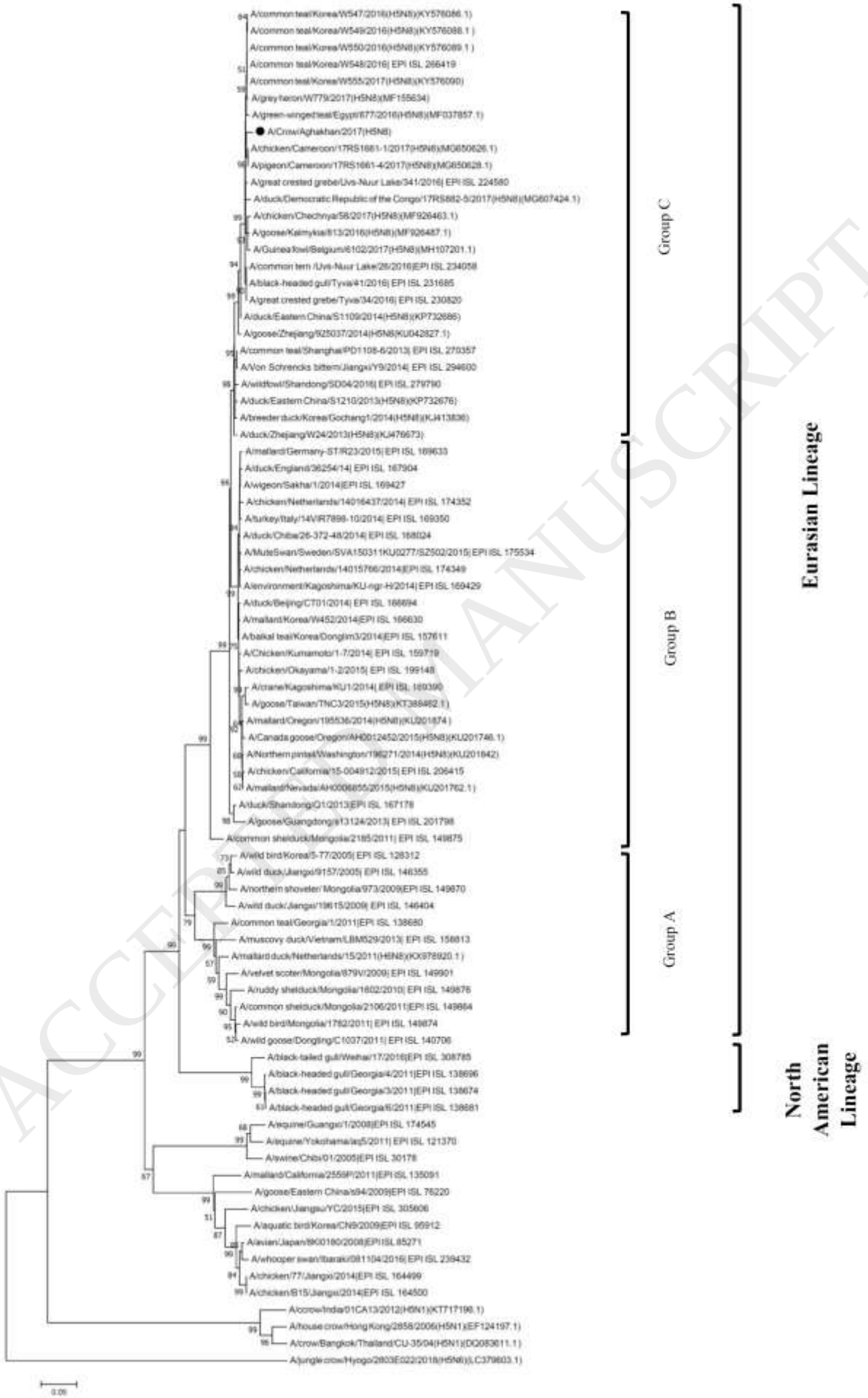
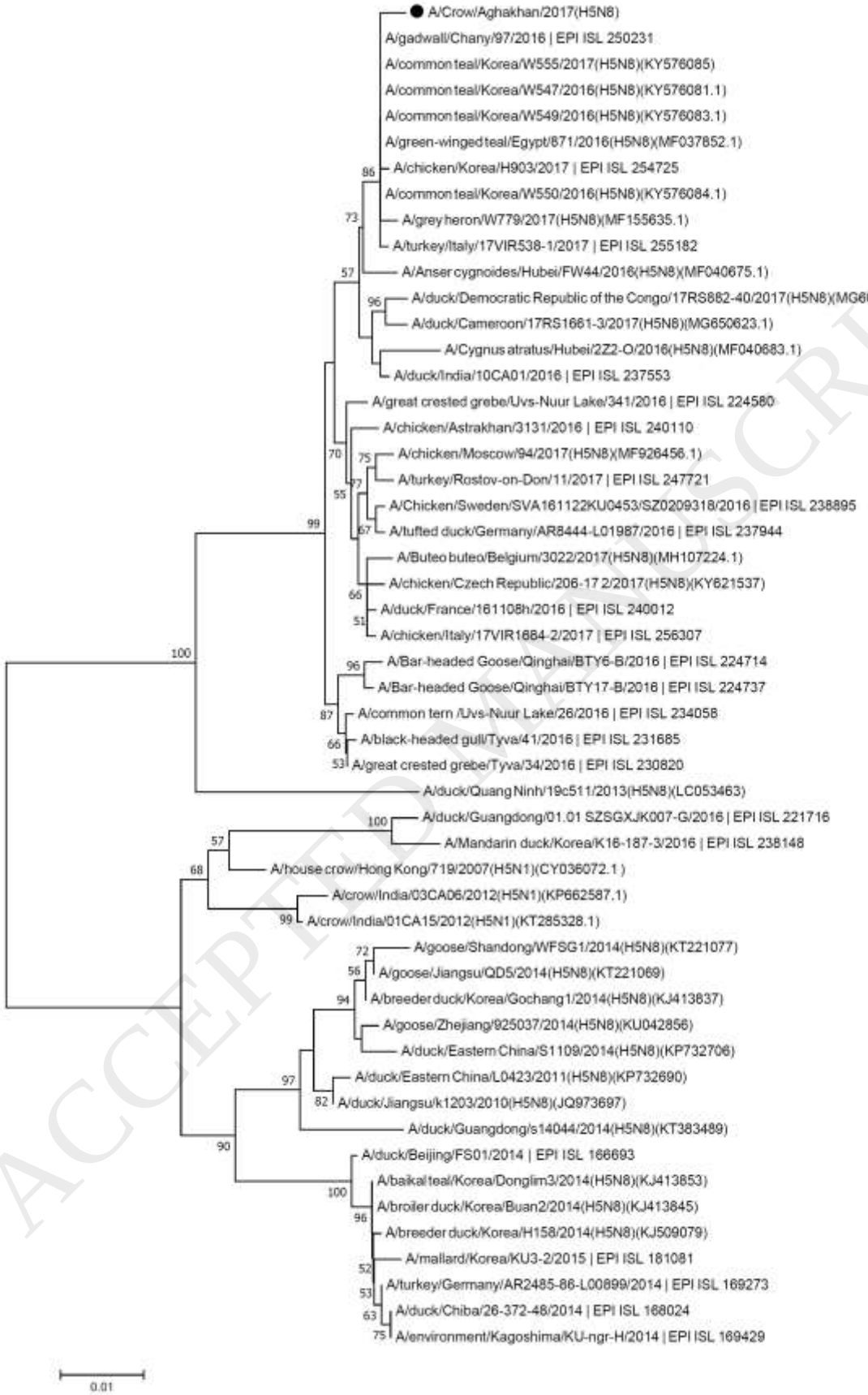
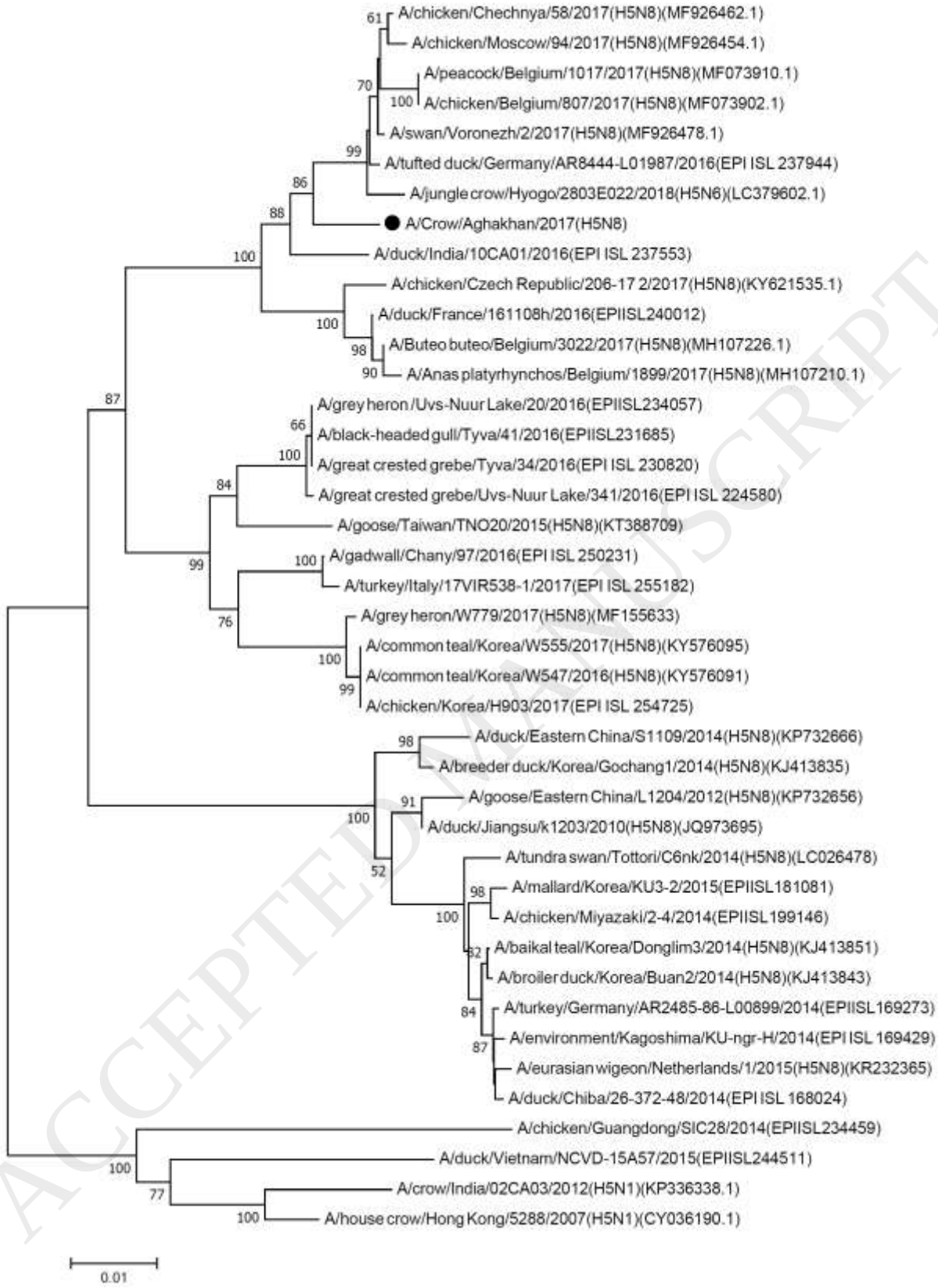


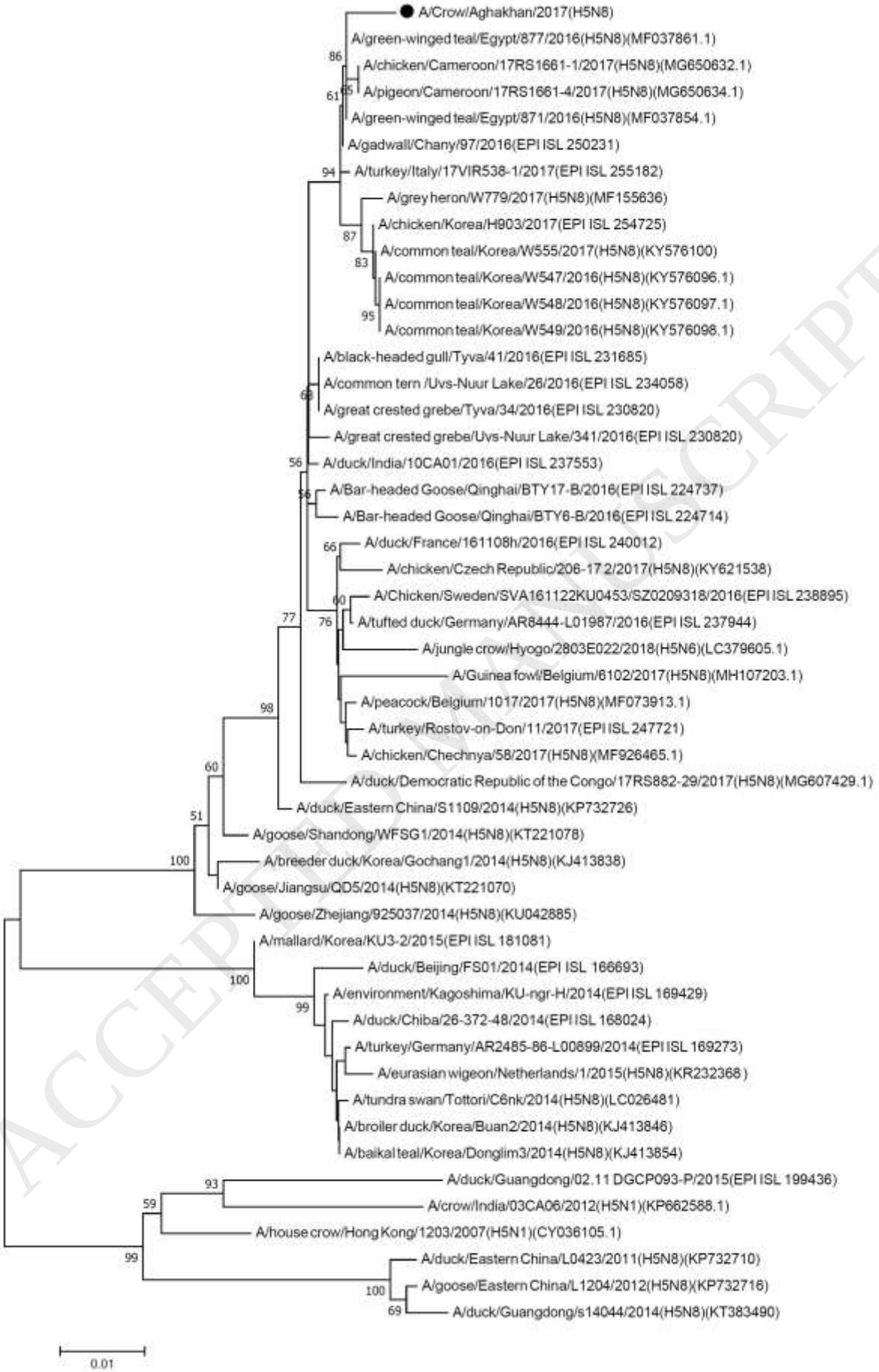
Fig 3. Phylogenetic tree of HA, NA, M, NP, NS, PA, PB1, PB2 (I-VIII) of the H5N8 avian influenza virus detected in Iran, 2018. The phylogenetic tree was constructed, using MEGA version 7, by the neighbor-joining method with 1000 bootstrap replicates (bootstrap values are shown on the tree). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (black circle: Iranian H5N8 samples)

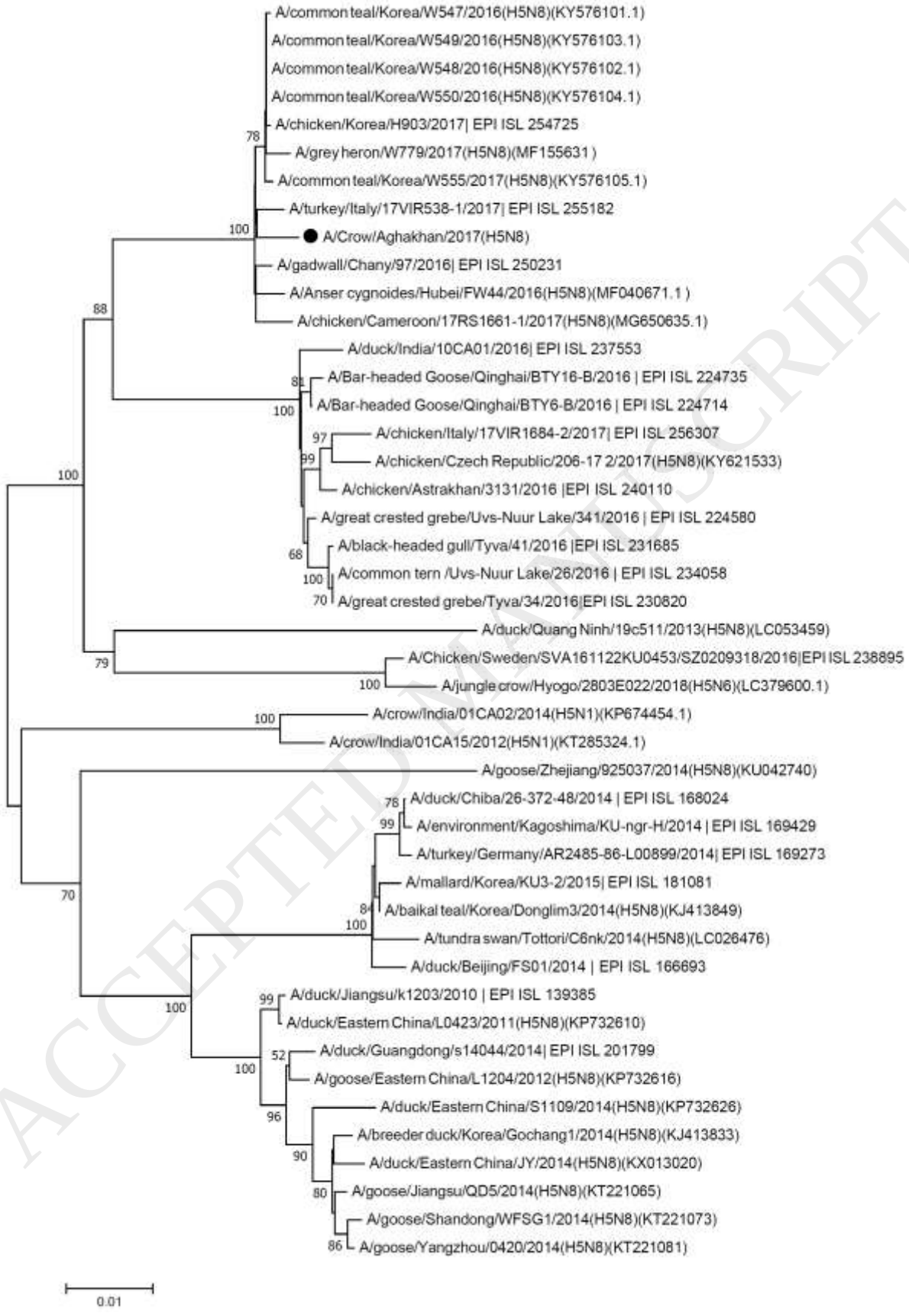


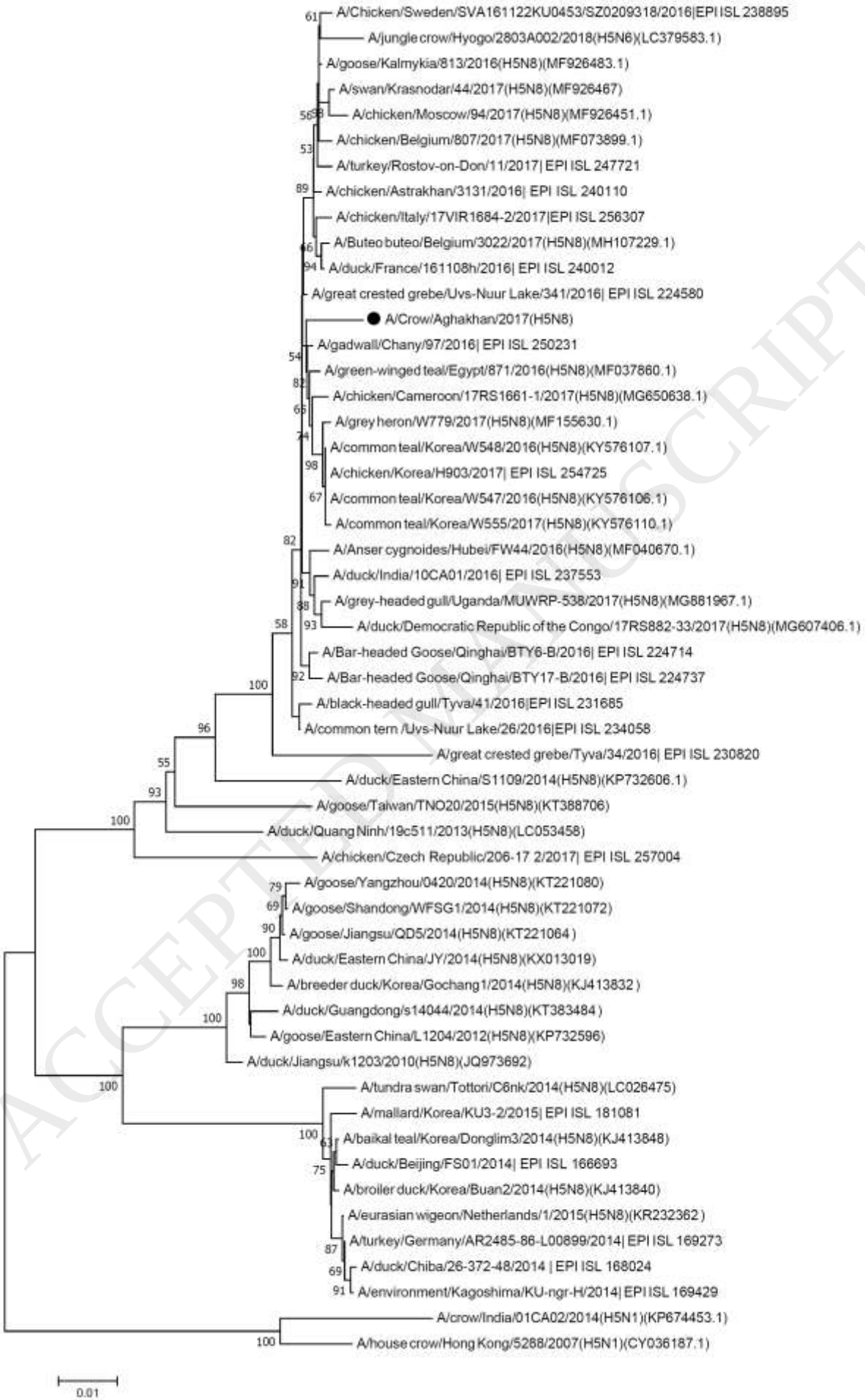












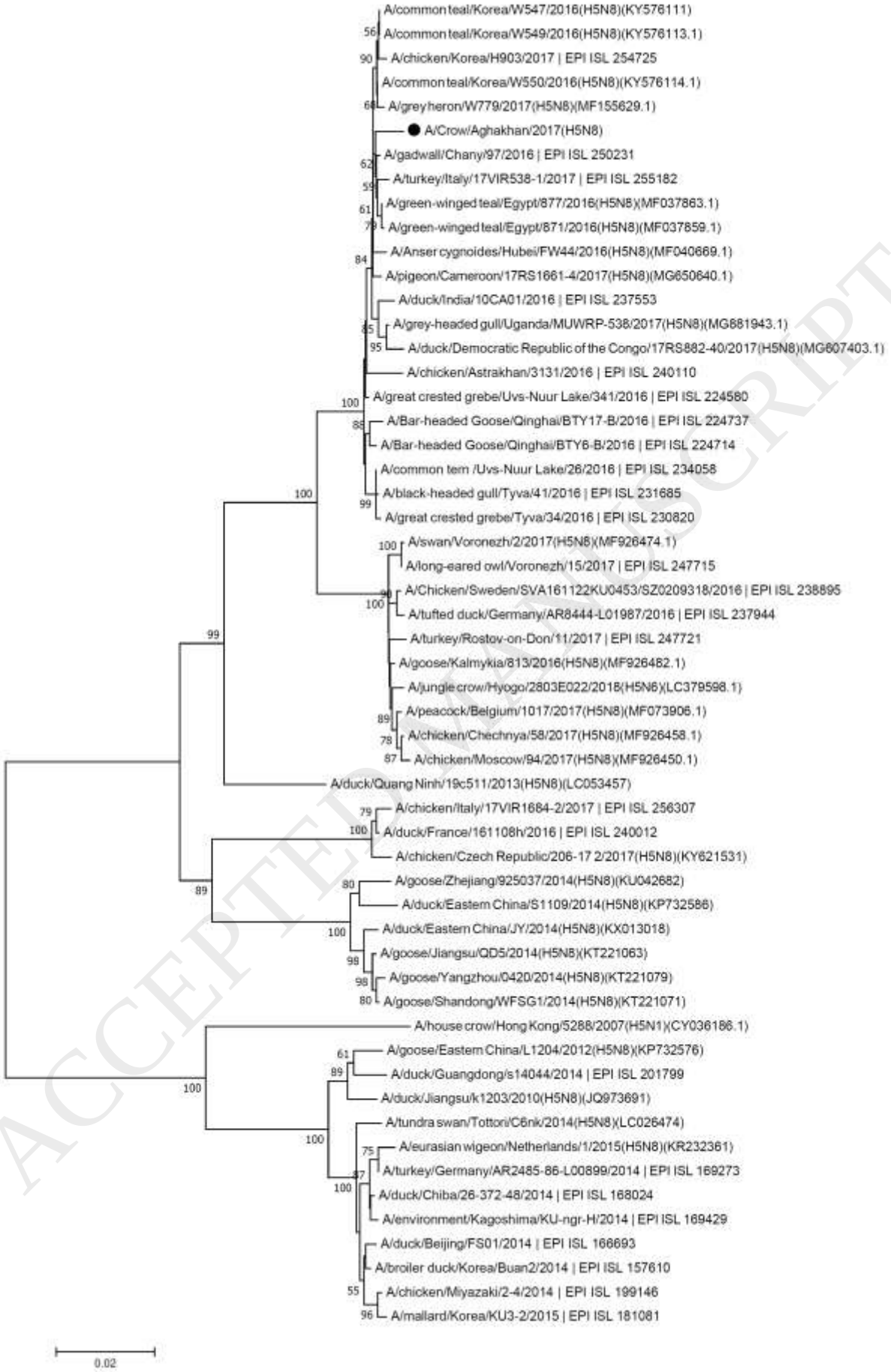


Table 1. Antigenic and receptor binding sites of Hemagglutinin protein of Aghakhan virus

Table 1: Antigenic and receptor binding sites of Hemagglutinin protein of Aghkhan virus

	Amino acid position	A/Crow/Iran/Aghakhan/2017	References
HA antigenic and receptor binding sites	138	A	[20]
	186	N	[37]
	196	K	[34]
	201	E	[19]
	222	Q	[28]
	224	N	[2]
	226	Q	[16], [39]
	228	G	[16]

Table 2. Nucleotide sequence homology of Aghakhan H5N8 HPAI with other H5N8 isolates.

Aghakhan	Maximum Identity with		
HA	Is-1076 (99.04%)	Ko-W549 (98.80%)	Cam-17RS1661-4 (98.80%)
NA	Cam-17RS1661-4 (98.84%)	Cam-17RS1661-1 (98.84%)	Ko-W555 (98.84%)
M	Ko-W555 (99.69%)	Eg-871 (99.69%)	Ko-W549 (99.69%)
NP	Vo-2 (98.41%)	Che-58 (98.22%)	Mo-94 (98.13%)
NS	Eg-871 (99.41%)	Cam-17RS1661-1 (99.26%)	It-17VIR538-1 (99.26%)
PA	Ko-W555 (99.38%)	Ko-W547 (99.34%)	It-17VIR538-1 (99.19%)
PB1	Cha-97 (98.92%)	Eg-871 (98.78%)	Ko-W548 (98.69%)
PB2	Ko-W550 (99.28%)	Eg-877 (99.28%)	Eg-871 (99.24%)

Aghakhan: A/Crow/Aghakhan/2017; Is-1076: A/turkey/Israel/1076/2016(H5N8); Ko-W549: A/common_teal/Korea/W549/2016(H5N8); Cam-17RS1661-4: A/pigeon/Cameroon/17RS1661-4/2017(H5N8); Cam-17RS1661-1: A/chicken/Cameroon/17RS1661-1/2017(H5N8); Ko-W555: A/common_teal/Korea/W555/2017(H5N8); Eg-871: A/green-winged_teal/Egypt/871/2016(H5N8); Ko-W549: A/common_teal/Korea/W549/2016(H5N8); Vo-2: A/swan/Voronezh/2/2017(H5N8); Che-58: A/chicken/Chechnya/58/2017(H5N8); Mo-94: A/chicken/Moscow/94/2017(H5N8); It-17VIR538-1: A/turkey/Italy/17VIR538-1/2017 (H5N8); Ko-W547: A/common_teal/Korea/W547/2016(H5N8); Cha-97: A/gadwall/Chany/97/2016 (H5N8); Ko-W548: A/common_teal/Korea/W548/2016(H5N8); Ko-W550: A/common_teal/Korea/W550/2016(H5N8); Eg-877: A/green-winged_teal/Egypt/877/2016(H5N8)