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LETTER

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Influenza A H9N2 is a low pathogenic virus present worldwide in domestic birds and, since the mid-1990s, it has been found to be endemic in poultry of southern China (Xu et al, 2007). Several cases of bird-to-human transmission have been reported in this area since 1999. Recently, Xu et al (2007) provided clear evidence that in southern China H9N2 and the highly-pathogenic H5N1 avian influenza viruses (AIV) have exchanged gene segments to generate currently circulating reassortants of both subtypes. Such genetic exchanges are likely to enhance the potential of pandemic spread of these viruses.

During investigation of AIV in wild birds in the Camargue (Rhône delta, South of France), one Mediterranean gull (*Larus melanocephalus*) was tested positive for H9N2 AIV infection by reverse transcription-polymerase chain reaction (Lebarbenchon et al, 2007). In gulls, AIV are divided into American and Eurasian lineages, and some specific subtypes (*i.e.,* H13, H16) are thought to be associated with the gull reservoir. In northern America, the presence of H9 AIV has been reported in shorebirds (including gulls) in combination with all neuraminidase (NA) subtypes except N3. In Europe H9 AIV have rarely been reported in wild birds (Munster et al, 2007) and has never been found in gull species. N2 is, however, the most frequent NA subtype in European waterfowl and a wide diversity of subtype combinations have been found in poultry. Here, we investigated the origin of the H9N2 AIV in Mediterranean gulls by sequencing 413 base pairs (bp) of the hemagglutinin (HA) and 453 bp of the neuraminidase (NA). We performed phylogenetic analysis including sequences from viruses isolated worldwide from wild birds (especially North American shorebirds) and domestic birds (Europe and southern China).

cDNA sequencing was done with the following set of primers: H9-151f: 5'-CTYCACACAGARCACAATGG-3' and H9-638r: 5'-GTCACACTTGTTGTTGTRTC-3' (Lee et al, 2001); NAN2-up3: 5'-CTTGTTGACAGTATT GGTTCATGGTCT-3' and NAN2-dw2: 5'-AAAGTCTC ATAACCTGAGCGAGAATC-3'; using Big Dye Terminator v.1.1 DNA sequencing kit in an Applied Biosystem 3700 automated sequencer. Alignments were performed using complete sequences available from the Influenza Sequence Database, using the progressive alignment algorithm implemented in CLC Free Workbench 4.0.2. Phylogenetic trees were reconstructed using maximum parsimony (MP) methods with the dnapars program of the PHYLIP 3.61 package and the maximum likelihood (ML) with the software PhyML 2.4.4. We used the GTR evolutionary model for the HA and the TN93 for the NA, as selected by Model Generator 0.84. Nucleotide heterogeneity and substitution rates were estimated with a gamma distribution (Γ). Nodal supports were assessed with 100 bootstrap replicates generated for each method.

The H9 sequence (EU333949) from our specimen is more related to that of American viruses isolated in 2003 from shorebirds (H9N1 and H9N5) than to H9N2 viruses present in Eurasian birds (Figure 1). Analysis of the NA sequence (EU333950) reveals however that the N2 sequence is closely-related to that of viruses isolated from European wild and domestic birds (H5N2, H6N2 and H9N2), and

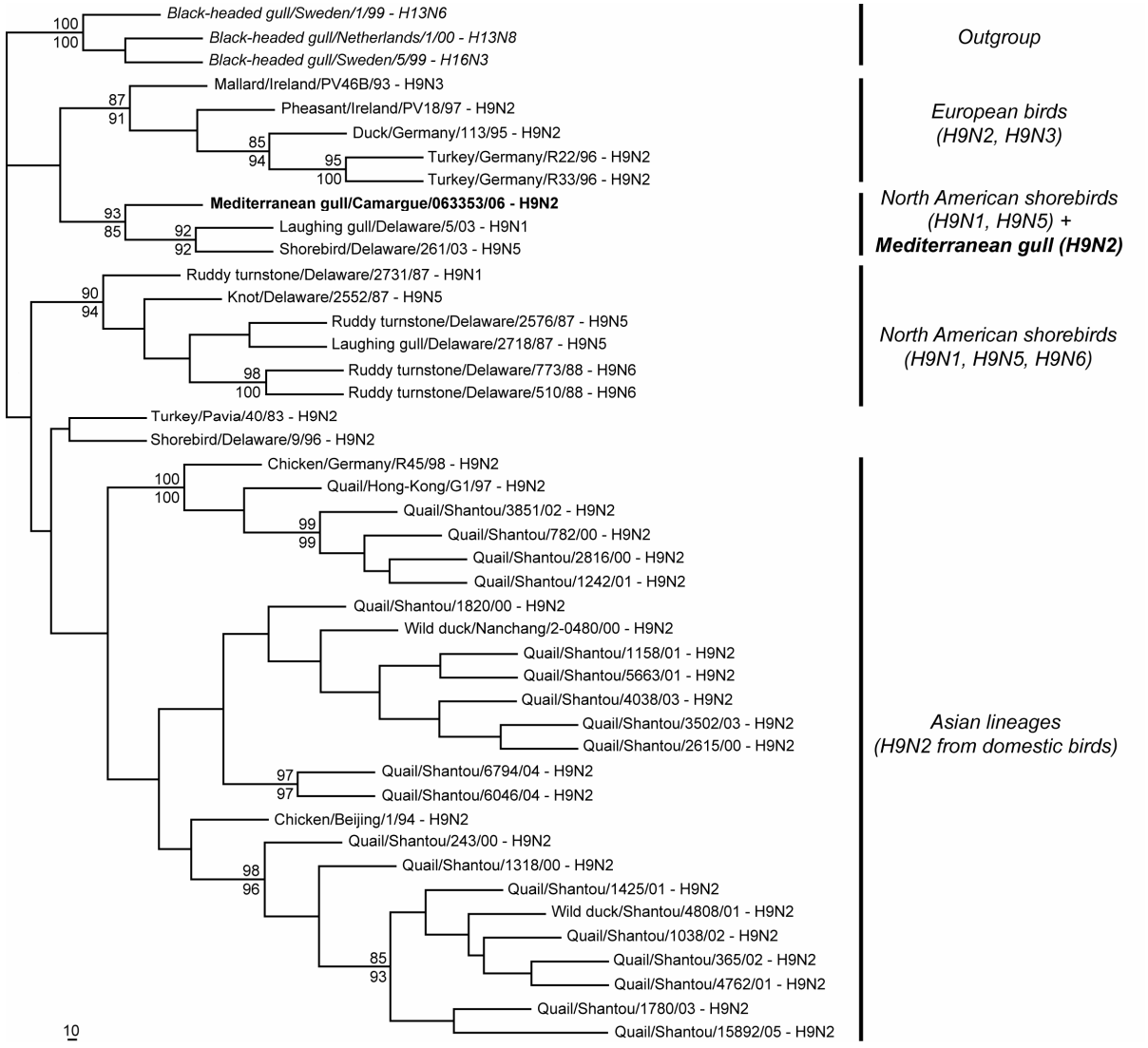

Figure 1. ML consensus phylogram trees for the HA partial gene sequences, using the GTR+Γ (α=0.42) evolutionary model. Bootstrap proportions calculated after 100 replications are indicated at nodes in MP (up) and ML (down). Only bootstrap values >90 in at least one of the two methods (MP and ML) are indicated.

clearly unrelated to that of viruses isolated from gulls in northern America (Figure 2).

These findings support different origins of the HA and NA segments for this virus. H9 is likely to result from natural exchanges of viruses between North American and European gull species. Mediterranean gulls are restricted to western Europe, making the possibility of a direct introduction of viruses belonging to the American H9 lineage by this species very unlikely. On the other hand, the occasional presence of North American gulls in France has been reported as small groups of the ring-billed gull (*Larus delawarensis*) cross the Atlantic Ocean each year. The black-headed gull (*Larus ridibundus*) is also likely to be the vector of influenza exchanges between western Europe, where it is widely represented, and the eastern coast of Canada where breeding colonies are present each summer. Investigations into AIV infections among these bird populations may be of particular interest to understand patterns of intercontinental virus exchange.

The NA segment presumably originated from viruses that circulate in European wild or domestic birds, suggesting that the H9N2 virus we identified results from a reassortment event between American and Eurasian lineages of AIV.

Only a few studies have related natural intercontinental exchanges and gene segment reassortment (*e.g.,* Krauss et al, 2007). Our report further emphasizes that such inter-continental exchanges can occur, as reported by Jackwood and Stallknecht (2007), and may result in the generation of new strains of AIV.

In Europe, H9N2 AIV is rarely detected in wild birds (Munster et al, 2007), suggesting that its circulation is

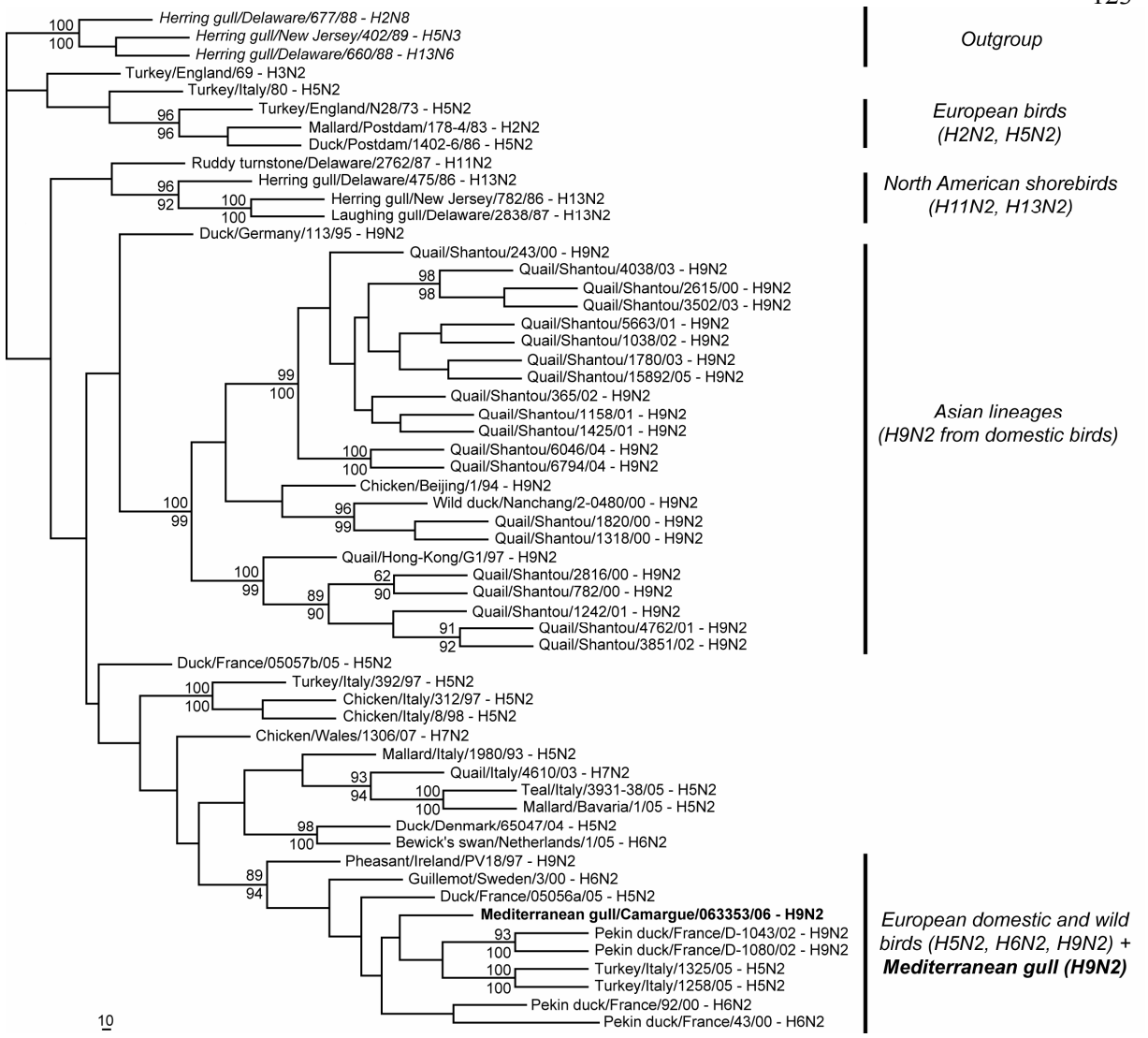

Figure 2. ML consensus phylogram trees for the NA partial gene sequences, using the TN93+Γ (α=0.34) evolutionary model. Bootstrap proportions calculated after 100 replications are indicated at nodes in MP (up) and ML (down). Only bootstrap values >90 in at least one of the two methods (MP and ML) are indicated.

probably very limited among these birds. This clearly contrasts with the co-circulation of H9N2 lineages in poultry from Southern China that have a rapid and independent evolution (Figures 1 and 2) and raises questions as to the generation and emergence of potentially pandemic strains.

COMPETING INTERESTS

None declared.

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