The identification of a new genotype of avian paramyxoviruses type I in West-Africa provides new outcomes for phylogeny reconstruction

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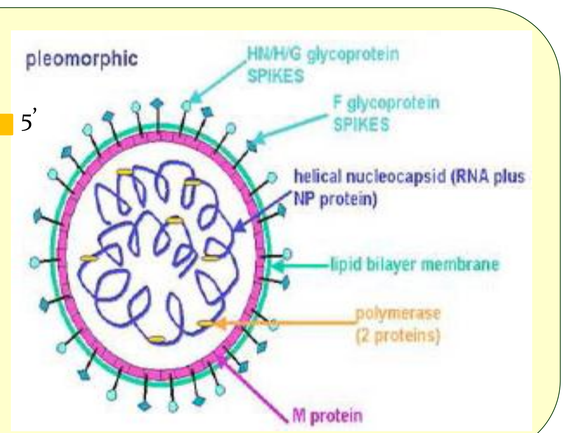
INTRODUCTION

Newcastle disease (ND) is one of the most severe infectious diseases of birds, particularly poultry, and has been the cause of serious economic losses worldwide. Newcastle disease virus (NDV) or avian paramyxovirus type 1 (APMV-1) are divided in two clades (class I and class II) according to the genome size as well as sequence of the F and L genes. Virulent strains are found in the class II. There is no clear consensus for the NDV class II taxonomy since some authors use genotypes [1] whereas others use lineages, both covering distinct isolate clusters. Ten genotypes were traditionally considered for class II but a 11th genotype was recently proposed by our group. Aldous et al. [2] proposed 6 phylogenetic lineages based on the topology of the maximum likelihood tree of the 375bp of the 3' end of the F gene (further subdivided into 13 sublineages) to replace the genotype nomenclature previously adopted [1].

In many parts of Africa, ND is considered as endemic but only few data are available on the variants involved . In West Africa, new subgenotypes VIIf, VIIg and VIIh were recently described [3] and integrated into the lineage 5 described by Aldous et al. [2]. Based on phylogenetic analyses of a partial F coding sequence of NDV isolates recovered from apparently healthy poultries in Mali in 2007 and 2008, we previously proposed a new subgenotype VIIi [4]. Cattoli et al [5] have also described new strains that were clustered into a new lineage 7 and four new sublineages, 7a, 7b, 7c and 7c, corresponding to our subgenotypes VIIi, VIIg, VIIh and VIIf, respectively.

Figure 1. schematic organisation of Newcastle disease virus genome and viral particle.
N: nucleocapsid, P: phosphoprotein, M: matrix, F: fusion, HN: hemagglutinin-neuraminidase, L: RNA-directed RNA polymerase. Two additional proteins (V and W) are encoded by the RNA editing of the P gene.



In this study we present and describe new NDV detected in West-Africa. Sequences from these viruses and others publicly available were included in an extensive phylogeny reconstruction with different methods. We propose a refined taxonomy for NDV genotyping showing better consistency than the former lineage nomenclature. Particularly, we introduce two new genotypes, XII and XIII using the analyses of the full length amino acids sequence of F and HN proteins.

MATERIALS AND METHODS

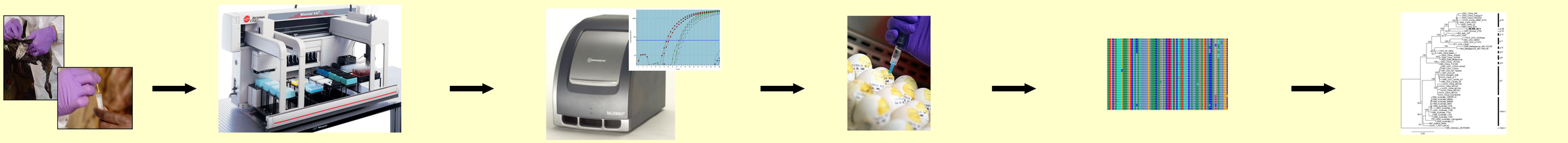


Figure 2. complete procedure for new NDV strains analysis

RESULTS

Ancient nomenclature	New nomenclature
Lineage 7	Genotype XIII
Genotype VIIc, d, e	Genotype XII
Genotype VIIb	Genotype VII

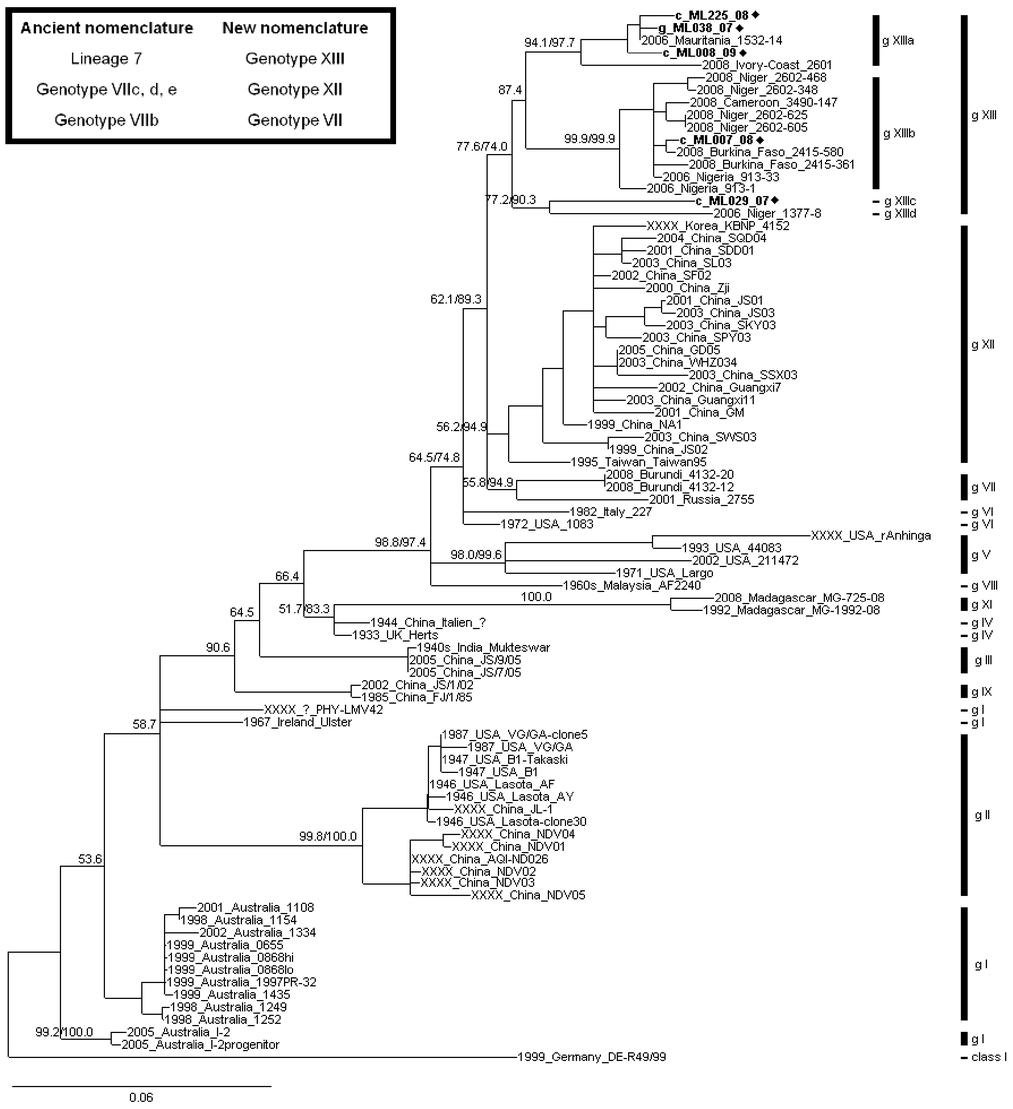


Figure 3. Phylogenetic analysis of 85 complete F and HN protein sequences of APMV-1. Trees were constructed using the maximum likelihood method with 1000 resampling for the bootstrap analysis. A class 1 strain was used as an outgroup. The F and HN trees were identical, thus, only the F tree is represented, where informative, branch support values for HN are indicated after the F bootstrap values. Sequences generated by our group are indicated with a parallelogram.

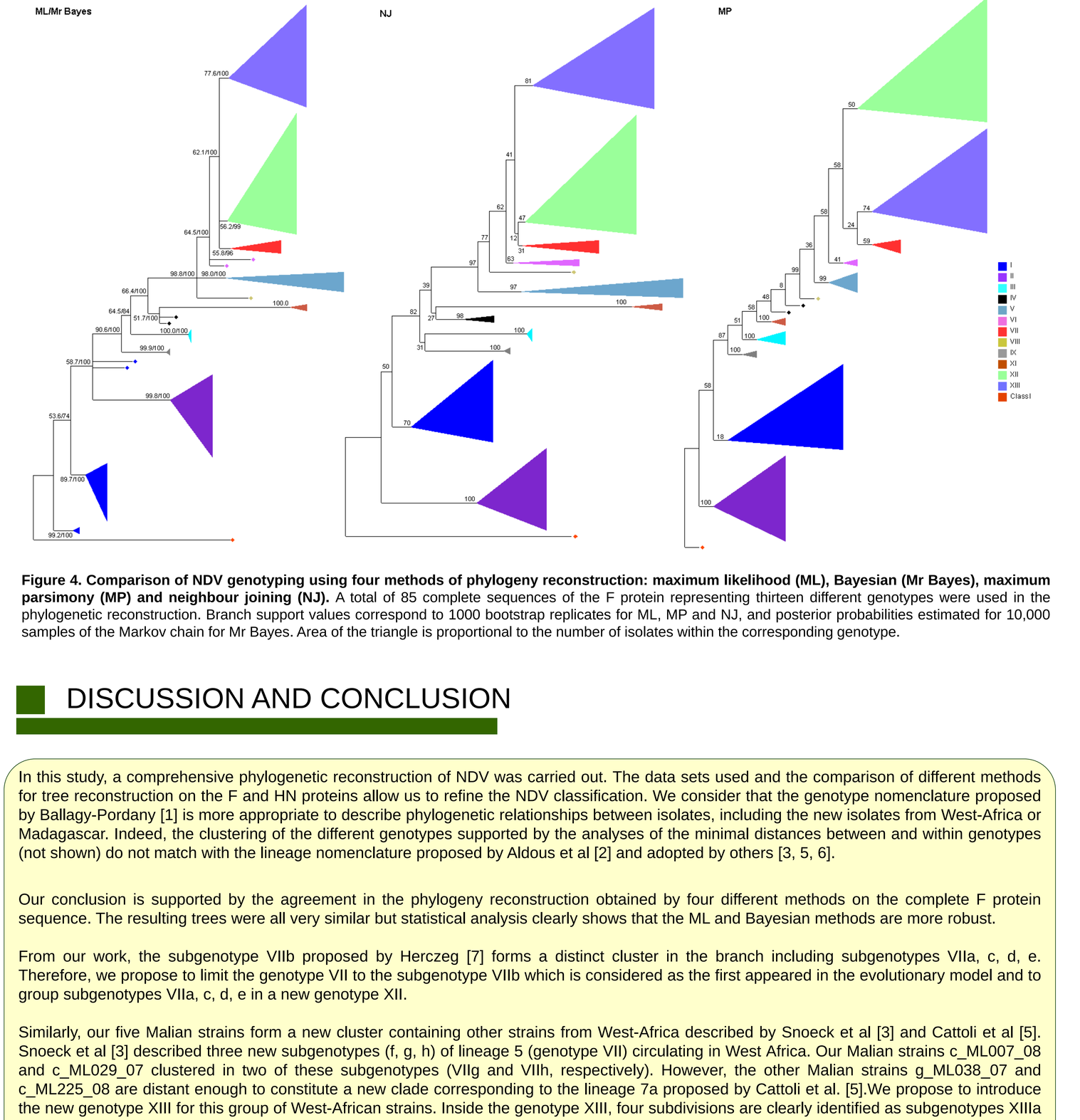


Figure 4. Comparison of NDV genotyping using four methods of phylogeny reconstruction: maximum likelihood (ML), Bayesian (Mr Bayes), maximum parsimony (MP) and neighbour joining (NJ). A total of 85 complete sequences of the F protein representing thirteen different genotypes were used in the phylogenetic reconstruction. Branch support values correspond to 1000 bootstrap replicates for ML, MP and NJ, and posterior probabilities estimated for 10,000 samples of the Markov chain for Mr Bayes. Area of the triangle is proportional to the number of isolates within the corresponding genotype.

DISCUSSION AND CONCLUSION

In this study, a comprehensive phylogenetic reconstruction of NDV was carried out. The data sets used and the comparison of different methods for tree reconstruction on the F and HN proteins allow us to refine the NDV classification. We consider that the genotype nomenclature proposed by Ballagy-Pordany [1] is more appropriate to describe phylogenetic relationships between isolates, including the new isolates from West-Africa or Madagascar. Indeed, the clustering of the different genotypes supported by the analyses of the minimal distances between and within genotypes (not shown) do not match with the lineage nomenclature proposed by Aldous et al [2] and adopted by others [3, 5, 6].

Our conclusion is supported by the agreement in the phylogeny reconstruction obtained by four different methods on the complete F protein sequence. The resulting trees were all very similar but statistical analysis clearly shows that the ML and Bayesian methods are more robust.

From our work, the subgenotype VIIb proposed by Herczeg [7] forms a distinct cluster in the branch including subgenotypes VIIa, c, d, e. Therefore, we propose to limit the genotype VII to the subgenotype VIIb which is considered as the first appeared in the evolutionary model and to group subgenotypes VIIa, c, d, e in a new genotype XII.

Similarly, our five Malian strains form a new cluster containing other strains from West-Africa described by Snoeck et al [3] and Cattoli et al [5]. Snoeck et al [3] described three new subgenotypes (f, g, h) of lineage 5 (genotype VII) circulating in West Africa. Our Malian strains c_ML007_08 and c_ML029_07 clustered in two of these subgenotypes (VIIg and VIIh, respectively). However, the other Malian strains g_ML038_07 and c_ML225_08 are distant enough to constitute a new clade corresponding to the lineage 7a proposed by Cattoli et al. [5].We propose to introduce the new genotype XIII for this group of West-African strains. Inside the genotype XIII, four subdivisions are clearly identified as subgenotypes XIIIa (ex lineage 7a), XIIIb (ex subgenotype VIIg), XIIIc (ex subgenotype VIIh) and XIIId (ex subgenotype VIIf).

This phylogenetic analysis suggests that this genotype XIII has evolved from a common ancestor with genotype VII (previously named subgenotype VIIb) and XII (previously named VIIc, d, e) but in a different direction than described earlier [3,5]. This suggests that an original strain older than genotype VII, probably linked to genotype VI was introduced in West Africa and since then has evolved in an independent manner to give genotypes VII and XIII whereas another lineage led to subgenotypes XII mainly found in Asia.

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