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RAPID COMMUNICATION

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Identification of a divergent genotype of equine arteritis virus from South American donkeys

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Summary

A novel equine arteritis virus (EAV) was isolated and sequenced from feral donkeys in Chile. Phylogenetic analysis indicates that the new virus and South African asinine strains diverged at least 100 years from equine EAV strains. The results indicate that asinine strains belonged to a different EAV genotype.

KEYWORDS

donkey, equine, equine arteritis virus, equine viral arteritis

1 | INTRODUCTION

Equine viral arteritis (EVA) is a viral disease in equids, namely horses, donkeys, mules and zebras. The causative agent is the equine arteritis virus (EAV), genus *Equartevirus* from the *Arteriviridae* family (Adams et al., 2017). EAV strains have been classified based on the ORF5 phylogeny into three genotypes, the North American (NA) and the European 1 (EU1) and 2 (EU2) lineages (Zhang et al., 2007).

Clinical disease is characterized by fever and respiratory symptoms; however, economic losses are mostly due to its ability to cause abortion in mares and severe disease or death in young foals (Balasuriya, Go, & MacLachlan, 2013). EAV increased global reporting during more recent years has been attributed to more frequent international horse movement (Dominguez, Münstermann, de Guindos, & Timoney, 2016). EVA is not only transmitted through direct contact during clinical respiratory disease, but it can also be transmitted through the venereal route. Stallions can become persistently infected (carriers) and transmit the disease during breeding (Guthrie et al., 2003).

2 | MATERIALS AND METHODS

In Chile, the EAV has not been detected in horses. In 2013, during surveillance activities, samples collected from feral donkeys ranging in small herds in hills and plains nearby the Atacama Desert were positive to neutralizing antibodies against EAV (Moreira, García, Valencia, & Moreno, 2016). Following results from this study, two male adult donkeys, clinically healthy, were captured in the annual rodeo event in October 2013. The rodeo was conducted at Carrizalillo, Freirina, Chile (−29.099469, −71.406169). Donkeys were sent to a slaughterhouse for human consumption.

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2.1 | Sample collection

Tissue samples including heart, lung, kidney, testes, vas deferens, epididymis, prostate and seminal vesicle were collected. One gram from each organ was scraped and homogenized with 10 ml of minimum essential media (MEM). The mix was centrifuged at 2,823 *g* for 20 min, and the supernatant was used for RT-PCR and virus isolation.

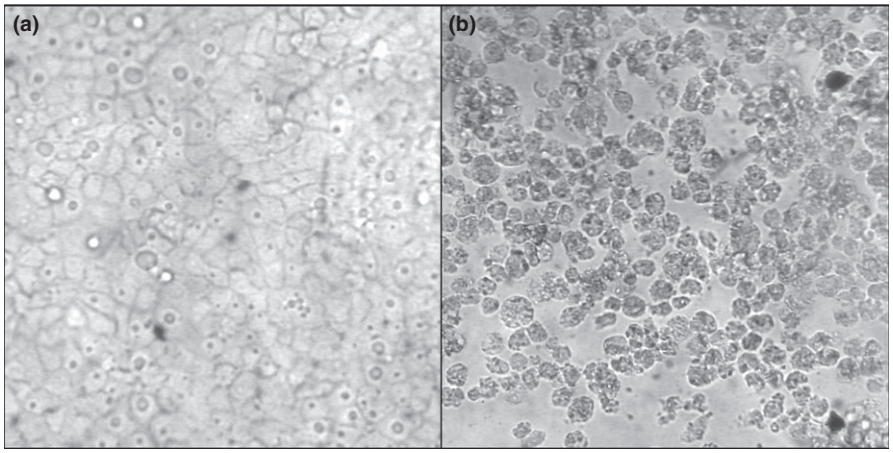

FIGURE 1 Cytopathogenic effects of RK-13 cells: (a) RK-13 cells mock-infected at 7 days post-inoculation. (b) RK-13 cells infected with Atacama-2014 equine arteritis virus (EAV) isolate at 7 days post-inoculation. [Colour figure can be viewed at wileyonlinelibrary.com]

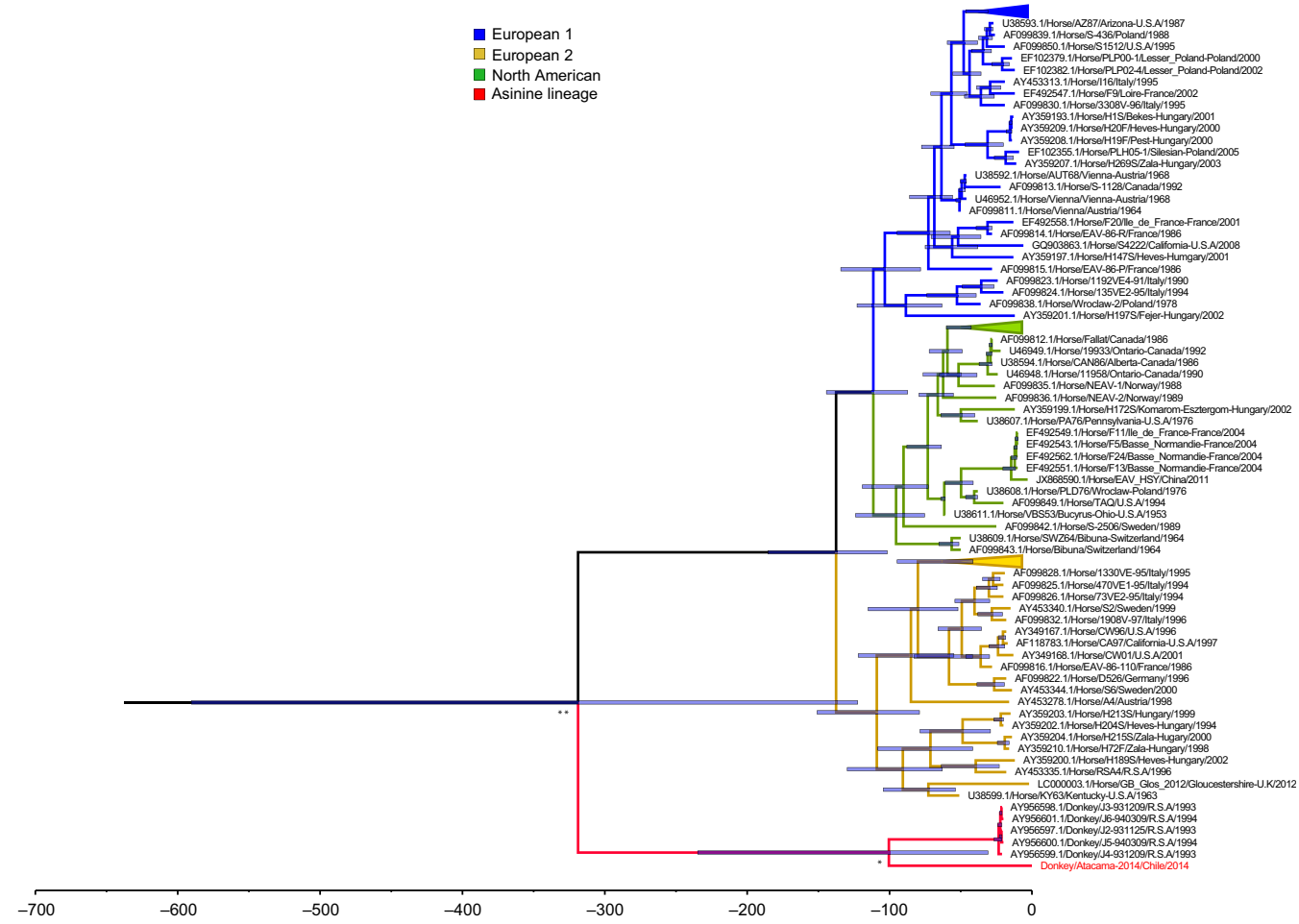

FIGURE 2 Maximum clade credibility collapsed tree of equine arteritis virus using 170 ORF5 reference sequences. The Atacama-2014 and South African Donkey sequences belong to a single monophyletic group, the asinine cluster (red). The time to most recent common ancestor (tMRCA) of the asisine cluster with other equine arteritis virus (EAV) sequences and the tMRCA of Atacama-2014 with the closest reference are indicated with * and **, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

2.2 | RT-PCR

RNA was extracted using the commercial kit MagMAX™-96 AI/ND Viral RNA Isolation Kit (Ambion® Cat# AM1835, Austin, TX, USA). ORF6 and ORF7 were amplified by RT-PCR using the protocols recommended by the World Organisation for Animal Health (Timoney, 2012). ORF5 was amplified using the primers and protocols previously described (Stadejek et al., 1999). PCR products were submitted for Sanger sequencing. The positive samples were selected for virus isolation.

2.3 | Viral isolation

Viral isolation was attempted in RT-PCR-positive samples. First, monolayers of RK-13 cells (ATCC® CCL-37™) were grown in 12-well plates with cell growth media, which includes minimum essential medium Eagle's (MEM), supplemented with 10% foetal bovine serum, 10,000 IU/ml penicillin (1%), 10,000 μg/ml streptomycin (1%) and 25 μg/ml amphotericin B (1%). Monolayers with 80% of cell confluence were inoculated with 200 μl of filtrated positive samples and incubated for 1 hr at 37°C and 5% CO_2. After the incubation, the inoculum was discarded and cells were incubated for 10 days using cell growth medium previously described. The monolayers were observed daily during 10 days inspecting for evidence of cytopathogenic effects. Positive cultures were tested by RT-PCR to confirm the presence of the EAV.

2.4 | Phylogeny

ORF5 was used to reconstruct the EAV phylogeny. ORF5 is the most variable region of the virus and commonly used for EAV phylogeny. ORF5 sequence generated for this study and reference sequences covering the known spectrum of ORF5 genetic diversity were aligned using MUSCLE (Edgar, 2004).The codon partition and nucleotide substitution model was selected using partition finder based on the Bayesian information criterion (BIC) (Lanfear, Calcott, Ho, & Guindon, 2012). The best scheme consisted in one partition for each codon position: HKY+I+G for codon position 1, TrN+I+G for codon position 2 and GTR+I+G for codon position 3. We used a Bayesian approach for time divergence estimation implemented in BEAST 1.8.2 (Drummond & Rambaut, 2007). Initially, a strict clock model and an uncorrelated relaxed lognormal clock, in combination

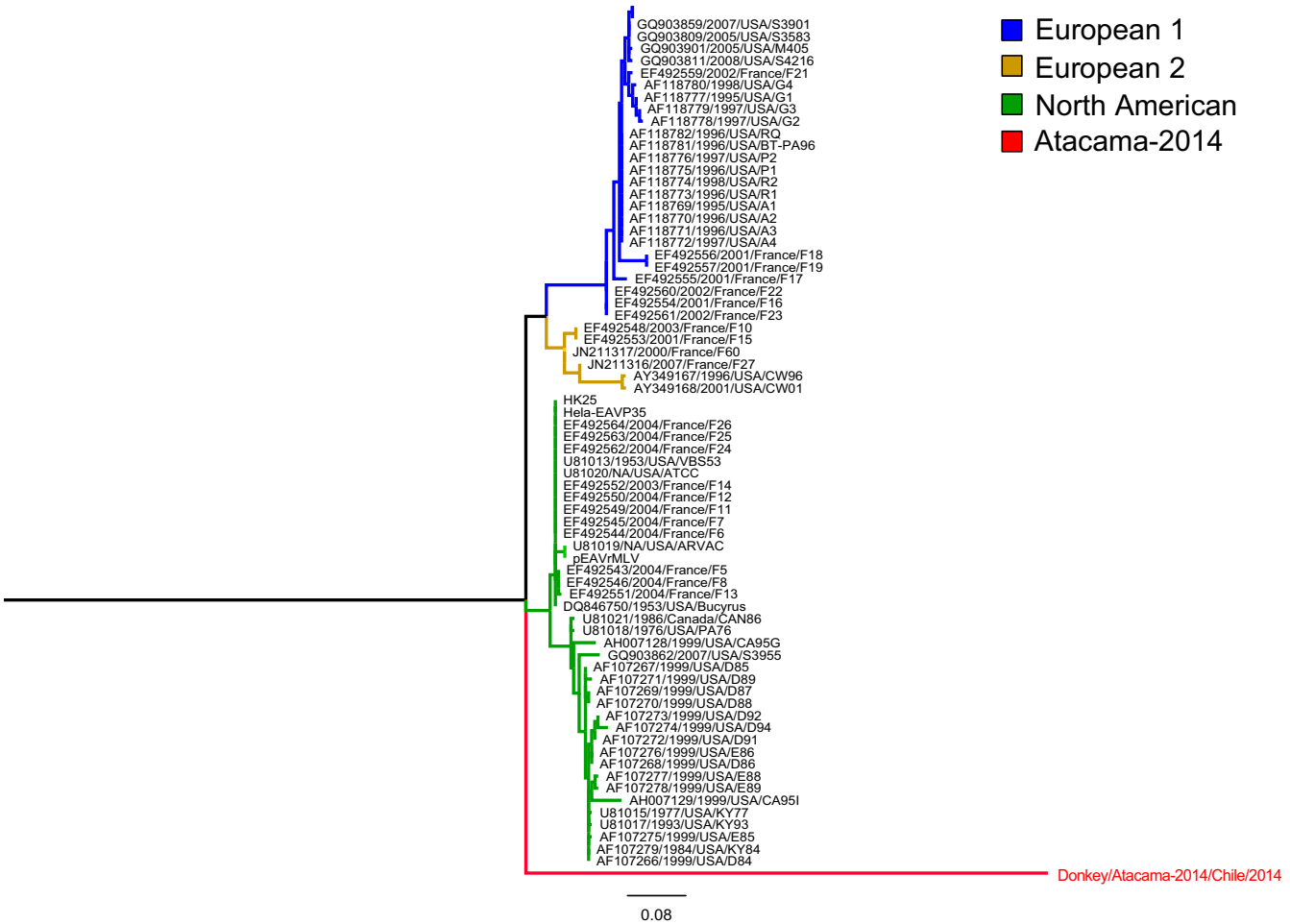

FIGURE 3 Maximum likelihood ORF6 phylogenetic tree using 93 equine arteritis virus reference sequences. Atacama-2014 isolate is a singleton genetically distant from reference sequences coloured in red. [Colour figure can be viewed at wileyonlinelibrary.com]

with a constant population and a Bayesian skyline tree prior, were run. We selected the model based upon the AICm method-of-moments estimator (Baele et al., 2012; Raftery, Newton, Satagopan, & Krivitsky, 2007) implemented in Tracer v1.6 (Rambaut, Suchard, Xie, & Drummond, 2014). Based on the lower AICm, the uncorrelated exponential clock model and a coalescent constant population tree prior were selected. The analysis was run for 200,000 iterations. Convergence and mixing of the simulations was assessed using Tracer. The maximum clade credibility tree (MCC) was visualized in Figtree version 1.4.2 (Rambaut, 2014). Additionally, phylogenetic analyses of ORF6 and ORF7, which are more conserved genes, were performed using MUSCLE for sequence alignment and maximum likelihood to reconstruct the phylogeny in MEGA7 (Kumar, Stecher, & Tamura, 2016).

3 | RESULTS AND DISCUSSION

Viral RNA was identified in samples from both animals. From a vas deferens sample, cytopathogenic effects (CPE) were observed at 5 days post-inoculation. CPE was characterized by rounding of cells and cell detachment from the monolayer (Figure 1). The isolated virus was named Atacama-2014.

Viral RNA was amplified, and partial sequences of ORF5 (630 nucleotides- nt), ORF6 (193 nt) and ORF7 (316 nt) were obtained by Sanger sequencing method (Accession numbers MF543058, MF543059 and MF573786, respectively). The phylogeny of ORF5 revealed that the Atacama-2014 belonged to a monophyletic cluster that included viruses collected from donkeys in 1993–1994 samples (Stadejek, Mittelholzer, Oleksiewicz, Paweska, & Belák,

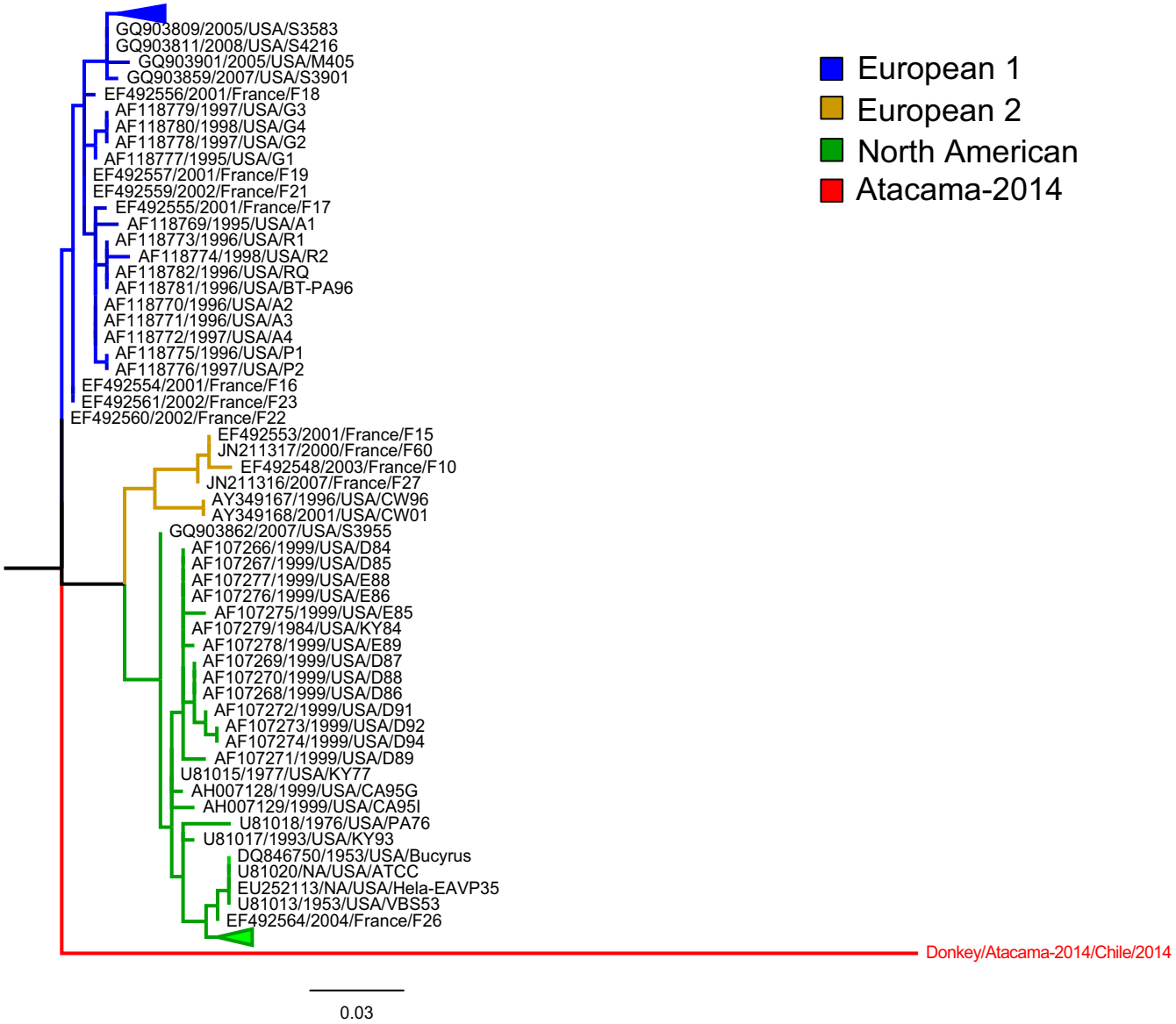

FIGURE 4 Maximum likelihood ORF7 phylogenetic tree using 93 equine arteritis virus reference sequences. Atacama-2014 isolate is a singleton genetically distant from reference sequences coloured in red. [Colour figure can be viewed at wileyonlinelibrary.com]

2006); we named this group the asinine cluster. The time to most recent common ancestor (tMRCA) between the asinine cluster and other EAV genotypes was estimated at 1695 (95% highest posterior density (HPD) interval 1424–1892) (Figure 2, Appendix S1). Additionally, the tMRCA of the Atacama-2014 sequence and the African donkey strains was estimated at 1914 (95% HPD 1779–1983). The ORF5 sequence with the highest identity to the Atacama-2014 virus (78.9%) that was public available was J2-931125 #AY9565 (South African asinine cluster). The ORF5 genetic distance between groups (NA, EU1, EU2 and asinine) was higher between the asinine group and all other clusters (79.5%–81.9%) compared to the remaining distances between the European and North American clusters (90.1%–89.1%). In South America, only EAV sequences from Argentina are available from public repositories. These Argentinean sequences were collected during the early 2000s and in 2011 and have been classified within the EU1 genotype, genetically distant to the asinine cluster (Metz, Serena, Panei, Nosetto, & Echeverria, 2014).

No ORF6 and ORF7 sequences from the donkey South African isolates were available for phylogenetic analysis. The ORF6 and ORF7 phylogeny shows the Atacama-2014 virus phylogeny as a singleton, distant from all other EAVs (Figures 3 and 4). However, we were able to sequence only three ORFs of the virus (10% genome), which we consider the major limitation of this study.

The phylogeny indicates that the asinine cluster represents a new genotype present in South America and Africa, both related to carrier donkeys. The presence of this genotype in two different continents may underlie a widely distributed unreported existence of this viral strain, different from the known and well-characterized EAV prevalent in horses.

It is not clear how the virus was introduced into the feral donkey population in Chile. Historical records indicate that the original population of donkeys arrived into the country at least 500 years ago. However, it is likely that subsequent importations occurred. tMRCA of the Chilean and 1993 South African donkey was estimated between 1779 and 1983, suggesting that the introduction of the virus may have occurred during imports after the original introduction of donkeys in Chile. However, because of the lack of availability of viral sequences collected at earlier time points of the asinine cluster, the tMRCA estimates should be carefully interpreted.

Although EAV has only been detected in donkeys in Chile, the South African asinine strains have also detected in horses (Stadejek et al., 2006); therefore, the EAV Chilean donkey viruses may represent a risk for different equine populations.

The characterization of this virus in South America provides a novel perspective of the global distribution of EAV. The isolation and genetic characterization of this new virus provides vital information for future EAV surveillance. It further contributes to understand the divergence of the virus and to the proper design of diagnostic test for more accurate detection in horses and as well as other equids.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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