

Short communication

Phylogenetic Analysis of the 16S rRNA gene of an *Anaplasma* sp. closely related to *Anaplasma phagocytophilum* from Cattle in Cebu, Philippines

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ABSTRACT

Anaplasma spp. are Gram-negative intracellular bacteria that can infect livestock animals and humans. In the Philippines, only *Anaplasma marginale* and *Anaplasma platys* have been widely reported. The zoonotic *Anaplasma phagocytophilum* has not been specifically detected yet. However, 16S rRNA fragments of *Anaplasma* spp. from cattle in Cebu, Philippines were detected and were found to be closest with *A. phagocytophilum*. This study aimed to further analyze these fragments. Phylogenetic analyses were performed using neighbor joining, maximum likelihood, and maximum parsimony. Phylogenetic analyses revealed that the obtained sequence consistently clustered with *A. phagocytophilum* regardless of the method used. Since *A. phagocytophilum* can infect humans, further studies must be conducted as the public dealing with livestock animals can be at risk.

KEYWORDS: 16S rRNA, *Anaplasma* spp., cattle, Cebu

Anaplasma spp. are gram negative intracellular rickettsial organisms that belong to the family *Anaplasmataceae* (Dumler *et al.*, 2001). They are mainly transmitted by ticks (Ybañez *et al.*, 2013a), although mechanical transmission is also possible (Scoles *et al.*, 2005). In the Philippines, specific molecular detections have been limited to *A. marginale*, *A. platys* and *A. centrale* (Ybañez *et al.*, 2013a; Ybañez *et al.*, 2013b).

A previous study (Ybañez *et al.*, 2013a) reported the molecular detection of DNA fragments (JX860284-6; 333-345 bp) of *Anaplasma* spp. in cattle in Cebu, Philippines, which were found to be closest with the zoonotic *Anaplasma phagocytophilum* (Dumler *et al.*, 2001). The former study only reported similarities and did not perform any further phylogenetic analyses. The present study aimed to phylogenetically analyze the obtained partial 16S rRNA gene sequences using three

different methods: neighbor-joining, maximum likelihood and maximum parsimony.

Phylogenetic analyses were performed as previously described (Ybañez *et al.*, 2016; Ybañez *et al.*, 2014). All analyses were performed using MEGA 7 (Kumar *et al.*, 2016, Stecher and Tamura, 2016), with *Rickettsia prowazekii* as the consistent outgroup. Neighbor joining (NJ) method used the maximum composite likelihood model, while the maximum likelihood (ML) method used the kimura-2 parameter model (based on the results of the best fit model).

Confirmation of the identity of the detected *Anaplasma* spp. is difficult because of its low 16S rRNA sequence similarity with established *Anaplasma* spp. Using 16S rRNA, at least 99.7% similarity is required before species can be confirmed (Ybañez *et al.*, 2012). However, the detected fragments were found closest with *A. phagocytophilum* (Ybañez *et al.*, 2012).

Phylogenetic analyses revealed that the obtained DNA fragments consistently grouped with *A. phagocytophilum* regardless of the methods used (Figure 1-3). Although with low bootstrap support, the detected sequences remain closest to *A. phagocytophilum*. As *A. phagocytophilum* is zoonotic and pathogenic, the detected bacteria can also have similar characteristics because of its close relationship. However, further studies must be conducted to clarify the epidemiologic status pathogenicity and identity of the detected *Anaplasma* spp. Other genes like the *gltA*, *ankA*, and *groESL* (Shukla *et al.*, 2007) can be explored.

The only known large ruminant tick in the Philippines is *Rhipicephalus microplus* (Ybañez *et al.*, 2012a). There is still lack of evidence that this can be a potential tick vector of *A. phagocytophilum*. Hence, the detection of DNA fragments that are found to be closely related to *A. phagocytophilum* in the Philippines must be clarified. It may also be a potentially new *Anaplasma* species that will require further genetic analysis and bacterial isolation to establish novelty (Ybañez *et al.*, 2012). On the other hand, Australia, which is one of the largest livestock trading partners of the Philippines, is also positive for *A. phagocytophilum* (Mayne, 2011). Hence, it is possible that *A. phagocytophilum* may have been brought by the

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p-ISSN: 2599-4875 e-ISSN: 2599-4980

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animals exported to the Philippines. The results of the present study do not only provide additional evidence on the genetic relatedness of the detected bacteria to *A.*

phagocytophilum but also calls for strict quarantine or screening of animals for pathogens that may affect the health of the local livestock.

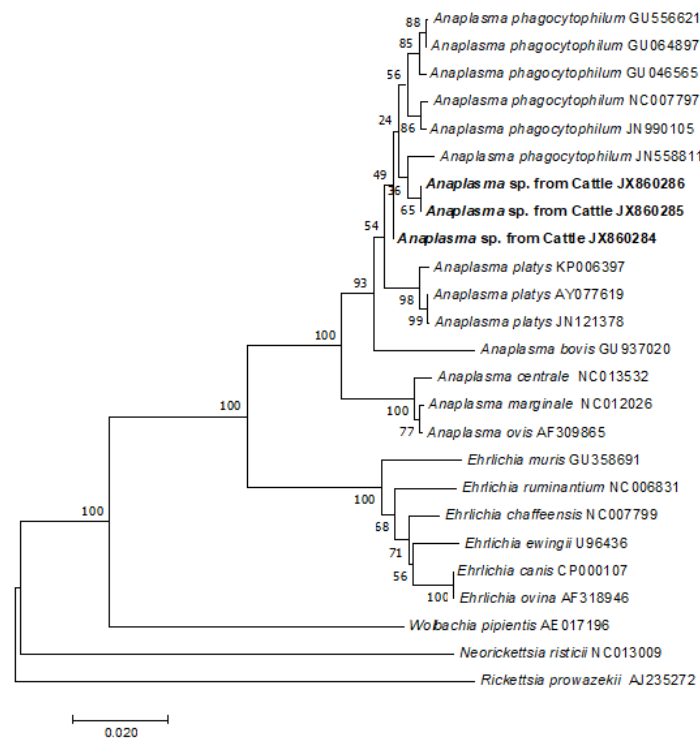


Figure 1. Phylogenetic tree analysis of an *Anaplasma* sp. from Cattle in Cebu, Philippines based on the 16S rRNA gene using the Neighbor-Joining method (Saitou and Nei, 1987) by the Maximum Composite Likelihood method (Tamura, Nei, and Kumar, 2004). *Rickettsia prowazekii* was set as the outgroup, and the sequence understudy is set in bold.

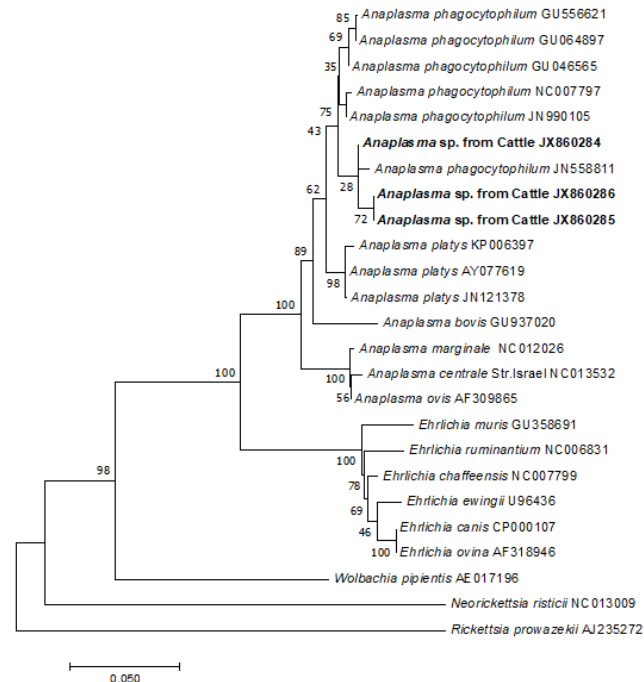


Figure 2. Phylogenetic tree analysis of an *Anaplasma* sp. from Cattle in Cebu, Philippines based on the 16S rRNA gene using the Maximum Likelihood method based on the Kimura 2-parameter model. *Rickettsia prowazekii* was set as the outgroup, and the sequence understudy is set in bold.

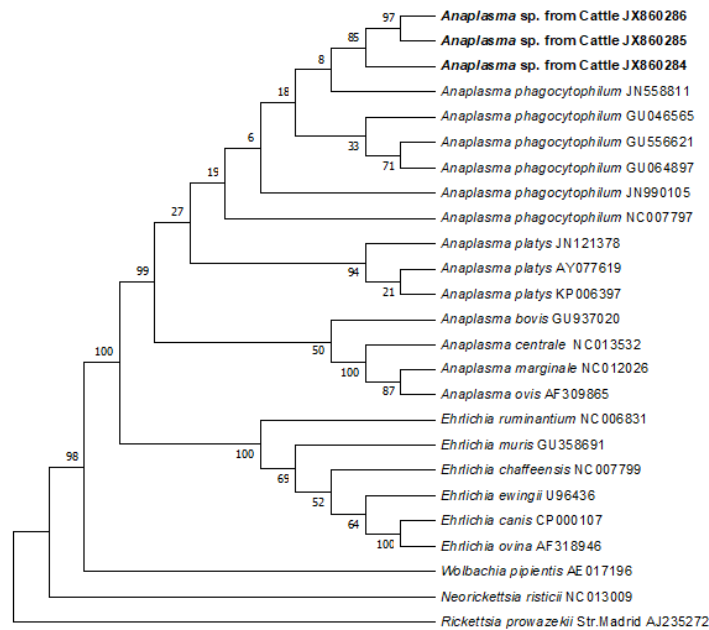


Figure 3. Phylogenetic tree analysis of an *Anaplasma* sp. from Cattle in Cebu, Philippines based on the 16S rRNA gene using the Maximum Parsimony (MP) method. *Rickettsia prowazekii* was set as the outgroup, and the sequence under study is set in bold.

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