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Zoonotic *Anaplasma platys* in Vietnamese family-kept dogs and dual infections with *Anaplasma marginale* in cattle[1]

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ABSTRACT.- Chien NTH, Nguyen KT, Le TH. **Zoonotic *Anaplasma platys* infections in Vietnamese family-kept dogs and dual infections with *Anaplasma marginale* in cattle**. *Pesquisa Veterinária Brasileira 46:e07796, 2026.* Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Ngo Xuan Quang Street, Gia Lam District, Hanoi, Vietnam. E-mail: mibtvn@gmail.com

Anaplasmosis is a widespread zoonotic tick-borne disease caused by *Anaplasma* species. This study analyzed 107 blood samples (68 canine and 39 bovine) stained using the Diff-Quik method for *Anaplasma* morulae. Microscopic evaluation and molecular validation via 16S rRNA gene sequencing identified *Anaplasma platys* in eight animals (three indigenous cattle, two hybrid dairy cows, and three indigenous dogs) and *Anaplasma marginale* in six cattle. Notably, one hybrid dairy cow showed dual infection with both *Anaplasma* species. The pairwise mean genetic distances were largest between *A. platys* and *Anaplasma capra* (4.30%) and lowest between A. platys and *Anaplasma phagocytophilum* (1.59%). Phylogenetic trees confirmed monophyletic *Anaplasma* and *Ehrlichia* clades, with Vietnamese *A. platys* isolates clustering as a sister group to the *A. phagocytophilum* subclade. The findings in our study confirm the persistent circulation of *A. platys* in Vietnam, highlighting its emerging zoonotic potential. Consequently, large-scale surveillance across animal hosts, vectors, and humans is essential to clarify the epidemiological status of Anaplasmataceae in the country.

INDEX TERMS: *Anaplasma platys*, *Anaplasma marginale*, morulae, genetic distance, 16S rRNA gene, phylogeny.

RESUMO.- [***Anaplasma platys* zoonótica em cães de criação familiar no Vietnã e infecções duplas por *Anaplasma marginale* em bovinos.**] A anaplasmose é uma zoonose disseminada transmitida por carrapatos, causada por espécies de *Anaplasma.* Este estudo analisou 107 amostras de sangue (68 caninas e 39 bovinas) para detecção de mórulas de *Anaplasma*. A validação molecular por meio do sequenciamento do gene 16S rRNA e a avaliação microscópica identificaram *Anaplasma platys* em oito animais (três bovinos nativos, duas vacas leiteiras mestiças e três cães nativos), e *Anaplasma marginale* em seis bovinos. Notavelmente, uma vaca leiteira mestiça apresentou infecção dupla por ambas as espécies. As distâncias genéticas médias entre pares foram maiores entre *A. platys* e *A. capra* (4,30%) e menores com *Anaplasma phagocytophilum* (1,59%). As árvores filogenéticas confirmaram os clados monofiléticos de *Anaplasma* e *Ehrlichia*, com os isolados vietnamitas de *A. platys* agrupando-se como um grupo irmão do subclado de *A. phagocytophilum*. Esses resultados confirmam a circulação persistente de *A. platys* no Vietnã, destacando seu risco zoonótico emergente e a necessidade urgente de medidas aprimoradas de diagnóstico e controle em animais domésticos.

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TERMOS DE INDEXAÇÃO: *Anaplasma platys*, *Anaplasma marginale*, bovinos, cães, distância genética, gene 16S rRNA, filogenia.

INTRODUCTION

The Anaplasmataceae family consists of four genera: *Anaplasma*, *Ehrlichia*, *Wolbachia*, and *Neorickettsia*, which are obligate, intracellular, Gram-negative, and pleomorphic bacteria (Dumler et al. 2001). Anaplasmosis is a tick-borne disease caused by *Anaplasma* species and is significant in tropical countries, where it is also recognized for its zoonotic importance (Huang et al. 2025). These pathogens, after entry, reside within membrane-bound phagosomes of neutrophils, monocytes, or lymphocytes, where they grow via binary fission to form characteristic mulberry-like inclusion bodies (Henniger et al. 2013, Choi et al. 2019, Rahamim et al. 2021). Globally, several species are recognized as zoonotic, including *Anaplasma phagocytophilum*, *Anaplasma platys*, *Anaplasma capra*, and occasionally *Anaplasma bovis* (Goel et al. 2018, Schudel et al. 2024, Huang et al. 2025, Nakao et al. 2025).

Anaplasma platys (formerly *Ehrlichia platys*) is a primary pathogen that infects canine platelets, resulting in infectious cyclic thrombocytopenia (ICCT). Notably, *A. platys* has zoonotic potential, capable of infecting humans and bovids, and can induce thrombocytopenia (low platelet count) (Al-Saadi et al. 2023, Arraga-Alvarado et al. 2024, Schudel et al. 2024). The presence of characteristic small, dark inclusions called "morulae" within platelets in a blood smear is the typical diagnostic criterion for detecting *A. platys* infection (Henniger et al. 2013, Choi et al. 2019). While most commonly associated with canine hosts, *A. platys* has been documented in cattle, potentially acting as a reservoir host, influencing the bacteria's transmission dynamics and genetic diversity (Chien et al. 2019, Kamani et al. 2022). *Anaplasma platys* has a wide geographical spread, confirmed in numerous case reports and studies worldwide, such as in Portugal (Cardoso et al. 2015, Pereira et al. 2016), Bulgaria (Gospodinova et al. 2024), Colombia (Vargas-Hernandez et al. 2016, Pesapane et al. 2019), Argentina (Maas et al. 2024), Brazil (Araújo et al. 2025), Mexico (Almazán et al. 2016), the Caribbean island of Saint Kitts (Alhassan et al. 2021), Egypt (Selim et al. 2021), India (Bhowmik et al. 2025), Iraq (Arif et al. 2024), Myanmar (Hmoon et al. 2021), Senegal (Zobba et al. 2022), Taiwan (Chao et al. 2024), Thailand (Sarker et al. 2021, Purisarn et al. 2022, Seerintra et al. 2023), the Philippines (Ybañez et al. 2016), Vietnam (Chien et al. 2019), and many African countries (Mnisi et al. 2026).

Anaplasma platys infections have been globally documented in various domestic and wild animals, including cats, ruminants, and camelids. Specific reports span Algeria (Chadi et al. 2024), Brazil (Lima et al. 2010), China (Zhou et al. 2023), Iran (Rassouli et al. 2020), Nigeria (Kamani et al. 2022), Thailand (Salakij et al. 2012, Nguyen et al. 2020), and Vietnam (Chien et al. 2019). Additionally, the pathogen has been identified in wild species such as red foxes and wild boars in Portugal (Cardoso et al. 2015, Pereira et al. 2016) and red deer in Egypt (Selim et al. 2021). Coinfection of *A. platys* with other Anaplasmataceae species, including *Ehrlichia canis* in dogs and other *Anaplasma* species such as *A. phagocytophilum, A. marginale* or *A. bovis*, and other tick-borne pathogens has been reported in Italy, Egypt, the Philippines, Thailand, China, Venezuela, Brazil, and other countries (Suksawat et al. 2001, Zobba et al. 2015, 2022, Ybañez et al. 2016, Al-Hosary et al. 2020, André et al. 2020, Purisarn et al. 2022, Ribeiro et al. 2023, Zhou et al. 2023). *Anaplasma platys* infects animals/humans via bites from *Rhipicephalus sanguineus* (brown dog tick), a *sensu stricto* competent vector for transmission, that is often identified in family dogs (Snellgrove et al. 2020, Chao et al. 2024, Do et al. 2024b, Maas et al. 2024).

In Vietnam, few molecular studies on Anaplasmataceae and Rickettsiaceae began in the early 2000s, primarily focusing on ticks. However, studies targeting *Anaplasma* species in domestic animals, particularly *A. platys*, remain scarce (Parola et al. 2003, Geurden et al. 2008, Chien et al. 2019, Do et al. 2024a, 2024b). Our 2016–2017 investigation was the most recent to confirm *A. platys* in dogs and cattle (Chien et al. 2019), and there have been no further molecular updates since then. This study aims to identify *A. platys* in household dogs and nearby livestock (cattle/cows) through morulae detection in blood smears and full-length 16S rRNA sequencing, thereby clarifying the taxonomic relationships among Anaplasmataceae species. This approach will facilitate precise molecular identification, phylogenetic assessment, and evaluation of the potential zoonotic risk to humans.

MATERIALS AND METHODS

Ethical approval. The study was approved by the Animal Ethics Committee of the Faculty of Veterinary Science of Vietnam National University of Agriculture (VNUA-AECFVS) under No. CARE-2025/03. Sample collection was implemented by the authors, the provincial veterinarians and technicians.

Blood sample collection and staining. Samples of 1–2 mL of whole blood were collected from each animal into vials containing ethylenediaminetetraacetic acid (EDTA) anticoagulant (Sigma-Aldrich Co. LLC, Saint Louis, Missouri, USA) from owned dogs and housed cattle/cows in three northern provinces of Vietnam (Ha Noi,

Hai Duong, and Ha Tinh): 2016–2017, 2021, and 2023–2025 (Table 1). Overall, 107 blood samples were collected from three animal groups with distinct management practices. These comprised 39 samples from cattle of indigenous breeds (12–15 months) grazed in open fields during the day and confined at night with occasional supplementary feeding, as well as hybrid dairy cows (2.5–3 years) housed in intensive stall confinement to produce daily milk. The remaining 68 samples were obtained from the dogs aged 6–12 months, including home pets, who were left uncontrolled on the premises of the livestock and dairy farms. The sampling regions were Ha Noi (21°01′11″ N/105°56′14″ E), Hai Duong (20°56′0″ N/106°19′0″ E), and Ha Tinh (18°25′54″18 N/105°43′48″ E) provinces, Vietnam. Blood smears were stained using the Diff-Quik method. Briefly, air-dried blood smears were dipped into methanol (Solution I), then into eosin (Solution II) several times, and methylene blue (Solution III), each for 5–10 dips/1 s per dip. Slides were rinsed with water and air-dried, then examined under light microscopy at magnification of x1,000. On stained blood smears, we examine inclusion bodies (bodies), also known as morulae, inside platelets (dogs and cattle) for *Anaplasma platys* and erythrocytes (near the membrane) of cattle for *Anaplasma marginale*.

Genomic DNA extraction and primers. DNA was extracted from morula-positive samples using the GeneJet DNA Purification Kit (Thermo Fisher Scientific, USA). Based on the GenBank 16S rRNA gene sequences, six primers targeting conserved regions of the Anaplasmataceae family were designed. These included an external flanking pair — UAEHF (5'-TCCTGGCTCAGAACGAACGC-3') and UAEHR (5'-GAGGTAATCCAGCCGCAGG-3') — and two internal pairs: AEHF1/AEHR1 (5'-TGGCAGACGGGTGAGTAATGC-3'/5'-GAGTTAAGCCAATTCCCATGGC-3') and AEHF2/AEHR2 (5'-AGGATTAGATACCCTGGTAGTCC-3'/5'-AGGCGGAGTGCTTAACGCG-3'). These primers were used for polymerase chain reaction (PCR), nested PCR, and amplicon sequencing.

PCR amplification. Mixtures of 50 µL were prepared using 25 µL of 2X DreamTaq PCR Master Mix (Thermo Fisher Scientific Inc., MA, USA), 3 µL of the DNA template (10 ng/µL), 2 µL of each primer (10 pmol/µL), 2 µL of dimethyl sulfoxide (DMSO), and 16 µL of water. Initiation was at 94 °C for 5 min, followed by 35 cycles consisting of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, and extension at 72 °C for 2 min, with a final extension at 72 °C for 10 min. PCR products (10 µL of each) were examined on a 1% agarose gel stained with ethidium bromide and visualized under UV light (Wealtec, Sparks, Nevada, USA). After purification using the GeneJET Gel Extraction Kit (ThermoFisher Scientific, USA), all PCR products (~1.5 kb) were sequenced by a commercial service.

Phylogenetic analysis. An alignment of 67 nucleotide sequences of the near full-length 16*S* rRNA gene (1,458–1,482 bp) of *Anaplasma* (39 sequences), *Ehrlichia* strains (26 sequences), and two *Rickettsia* species as an outgroup was performed and used to construct a phylogenetic tree. The alignment included eight *A. platys* and six *A. marginale* sequences from the current investigation (Table 1), 25 reference *Anaplasma*, including *A. bovis* (n = 2), *A. capra* (n = 2), *A. centrale* (n = 2), *A. marginale* (n = 5), *A. ovis* (n = 2), *A. phagocytophilum* (n = 3), and *A. platys* (n = 9); and 26 *Ehrlichia*, including *E. canis* (n = 13), *E. chaffeesis* (n = 1), *E. dumleri* (n = 1), *E. ewingii* (n = 1), *E. hainanensis* (n = 1), *E. khabarensis* (n = 1), *E. maerkangensis* (n = 1), *E. minasensis* (n = 1), *E. muris* (n = 1), *E. pampeana* (n = 1), *E. regneryi* (n = 1), *E. ruminantium* (n = 1), *E. shimanensis* (n = 1), and *E. walkeri* (n = 1). The nucleotide sequences were aligned using ClustalW, and the phylogenetic analysis was carried out in MEGA 11 (Tamura et al. 2021). The phylogenetic tree was constructed using the maximum-likelihood (ML) method with the general time-reversible GTR + F + I model (γ rate heterogeneity and a proportion of invariant sites) and 1,000 bootstrap replicates. The tree was exported in Newick (.nwk) format and visualized with the FigTree 1.4.4 program (Rambaut 2018).

Mean genetic distance estimation. Mean genetic distances were calculated between *Anaplasma* species. The 39 *Anaplasma* sequences were divided into seven groups based on species, and mean group pairwise genetic distances (GD) were computed with the MEGA 11 software. These included *A. marginale* (n = 11), *A. phagocytophilum* (n = 3), *A. ovis* (n = 2), *A. bovis* (n = 2), *A. centrale* (n = 2), *A. capra* (n = 2), and *A. platys* (n = 17). The GD estimate was done using the maximum composite likelihood (MCL) model in MEGA11 and 1,000 bootstrap resamplings (Tamura et al. 2021).

RESULTS

Microscopic examination of *Anaplasma* species

A total of 107 blood samples (68 canine and 39 bovine) were screened for the presence of *Anaplasma* morulae. Microscopic evaluation of blood smears identified *Anaplasma platys* morulae within the platelets of eight animals: three indigenous cattle, two hybrid dairy cows, and three indigenous dogs (Table 1). Additionally, *Anaplasma marginale* was detected in the erythrocytes of six cattle (three indigenous and three hybrid), typically localized along the host cell membrane (Fig. 1–3). Inclusion bodies appeared either singly or in multiples across both host species. Notably, *A. platys* morulae in canine platelets were larger and exhibited more frequent clustering than those observed in bovine hosts (Fig. 1 and 3). For molecular validation, near full-length 16S rRNA

gene sequences (1,458–1,486 bp) were obtained and deposited in GenBank (Table 1). Accession numbers are MH686048–MH686050, PX435401–PX435404, and PX485204 for *A. platys*, and MH686043 and PX435405–PX435409 for *A. marginale*. *Anaplasma platys* was identified in eight samples: four collected recently (2021–2025), three from previous research (Chien et al. 2019), and one from a dog via retrospective analysis of the 2016–2017 collection. Notably, a 3-year-old lactating hybrid dairy cow examined in 2023 was diagnosed to be dually infected with *A. platys* (in platelets) and *A. marginale* (in erythrocytes), which was followed by sequencing and molecular confirmation (Fig. 3, Table 1). Microscopic examination of blood smears revealed extensive invasion by *A. platys*, with single and multiple morulae densely packed inside the platelets (Fig. 3). In addition, *A. marginale* was detected in six cattle samples spanning from 2017 to 2025. The persistent emergence of *A. platys* underscores its continued presence in domestic animals, posing a zoonotic risk to humans living in adjacent households.

Generic and taxonomic phylogenetic relationships

Maximum likelihood analysis clearly distinguished *Anaplasma* and *Ehrlichia* as two monophyletic clades with robust nodal support (99% and 87%, respectively) (Fig. 4). Within the *Anaplasma* clade, 17 *A. platys* sequences formed a subclade (77% bootstrap support), where eight Vietnamese sequences clustered with isolates from China and the Philippines. This group further joined a monophyletic subclade containing isolates from South Africa, Germany, Italy, France, Japan, Zambia, and China. While five Vietnamese *A. marginale* sequences grouped with isolates from South Africa, Zimbabwe, China, the Philippines, and Australia, one sequence clustered with *Anaplasma ovis* and *Anaplasma centrale*. This nesting indicates that *A. marginale* shares a higher phylogenetic affinity with these species than with other *Anaplasma* members. Notably, the *Ehrlichia* clade exhibited higher taxonomic resolution and a more distinct monophyletic topology than *Anaplasma* (Fig. 4).

Genetic distance between *Anaplasma* congeners

Table 2 displays the pairwise mean genetic distances (GD) among *Anaplasma* species groups based on full-length 16S rRNA gene sequences. The GD between the *A. platys* group and other congeners ranged from 1.59% to 4.30%, with the lowest divergence observed against *Anaplasma phagocytophilum* (1.59%) and the highest against *Anaplasma capra* (4.30%). Across all groups, the minimum inter-group GD occurred between *A. centrale* and *A. ovis* (0.63%), while the maximum was recorded between *A. capra* and *Anaplasma bovis* (5.7%). These genetic relationships align with the phylogenetic topology in Figure 4: the low GD values for *A. platys*/*A. phagocytophilum* and *A. centrale*/*A. ovis* support their respective monophyletic pairings, whereas the higher divergence between *A. platys* and *A. capra* is consistent with their paraphyletic positions.

DISCUSSION

In this study, *Anaplasma platys* was identified in five canine and bovine blood samples collected from 2021 to 2025, with three additional cases identified through retrospective analysis of 2016–2017 data. Notably, we report the first documented case of *Anaplasma* dual infection in Vietnam, involving *A. platys* and *Anaplasma marginale* in a bovine host, characterized by visible morulae in infected blood cells. Furthermore, phylogenetic and genetic distance analyses were conducted to clarify the taxonomic relationships and interspecific divergence within the *Anaplasma* genus and its association with *Ehrlichia*.

Combining microscopy with molecular techniques enabled the definitive identification of intracellular rickettsial bacteria in dogs and cattle and the use of molecular markers, primarily 16S rRNA gene, singly or combined with other genetic markers such as the 60 kDa heat-shock protein gene (*groEL*; or HSP60), beta subunit of RNA polymerase (*rpoB*), citrate synthase (*gltA*), and major surface protein 4 gene (*mps4*) sequences for phylogenetic assessment appeared to clarify best their taxonomic and generic relationships (Dumler et al. 2001, Huggins et al. 2022, Araújo et al. 2025). Members of the Anaplasmataceae family, including *A. platys*, *Anaplasma phagocytophilum*, *A. marginale*, and *Ehrlichia canis*, typically form single or multiple inclusion bodies (morulae) within host blood cells such as platelets, neutrophils, erythrocytes, and lymphocytes. These dark-staining bodies serve as pathognomonic diagnostic markers for the Anaplasmataceae infections (Goel et al. 2018, Choi et al. 2019, Rahamim et al. 2021). Specifically, the abundance of morulae in canine platelets reflects the robust proliferation of *A. platys* (Henniger et al. 2013, Vargas-Hernandez et al. 2016, Choi et al. 2019).

In this study, blood smear examinations for morulae were performed prior to or concurrently with molecular assays. We identified individual infections of *A. platys* and *A. marginale*, as well as a molecularly confirmed coinfection in a single animal (Fig. 1–3). This cow, found to harbor both *Anaplasma* species, belonged to a household in a suburban Hanoi village situated near grassland. In this area, *Rhipicephalus sanguineus* (brown dog tick) was detected on local dogs, suggesting potential pathogen transmission among domestic animals (Do et al. 2024b). While coinfections of *A. platys* with various Anaplasmataceae or other tick-borne pathogens, and *A.*

marginale with piroplasms have been reported (Ybañez et al. 2016, Yang et al. 2019, André et al. 2020, Al-Hosary et al. 2020, Kamani et al. 2022, Garcia Ribeiro et al. 2023, Zhou et al. 2023), the specific dual infection of *A. platys* and *A. marginale* in bovine hosts appears to be a novel finding. Although Zhou et al. (2023) documented complex coinfections involving *A. phagocytophilum*, *Anaplasma bovis*, *Anaplasma ovis*, and *A. marginale* in Chinese livestock, our study is the first to identify this particular species pair in cattle. These findings underscore that cattle can harbor multiple *Anaplasma* species, potentially exacerbating the clinical severity of bovine anaplasmosis.

Anaplasma platys exhibited significant genetic divergence from most congeners (3.6–4.07%) but remained closely related to *A. phagocytophilum* (1.59%). This taxonomic proximity is reflected in their sister-clade positioning (Fig. 4). These genetic distance (GD) values align with the tree topology: low GD between *A. platys*/*A. phagocytophilum* and *A. centrale*/*A. ovis* confirms their respective monophyletic pairings. Conversely, the higher divergence between *A. platys* and *Anaplasma capra* supports a paraphyletic relationship, consistent with previous reports (Salakij et al. 2012, Ybañez et al. 2016, Vargas-Hernandez et al. 2016, Sarker et al. 2021, Zobba et al. 2022, Zhou et al. 2023).

The sequences of 16S RNA genes were the most common among other genetic markers (*groEL*, *rpoB*, *gltA*, and *mps4*) for Anaplasmataceae diagnosis, phylogenetic analysis, metabarcoding for differentiating tick-borne pathogens, and assessment of taxonomic relationships (Dumler et al. 2001, Parola et al. 2003, Tana-Hernández et al. 2017, Chien et al. 2019, Huggins et al. 2022, Caudill & Brayton, 2022, Al-Saadi et al. 2023, Alkathiri et al. 2024, White et al. 2025). A thorough and consistent strategy was used to arrange four legitimate genera, *Anaplasma*, *Ehrlichia*, *Wolbachia*, and *Neorickettsia*, into the family Anaplasmataceae using 16S rDNA-based taxonomy, which has been satisfactorily verified (Dumler et al. 2001). The 16S rRNA gene, hence, is a reliable marker for species identification, multiplex detection, and phylogenetic relationships of tick-borne pathogens (Suksawat et al. 2001, Ybañez et al. 2016, Tana-Hernández et al. 2017, Caudill & Brayton 2022, Alkathiri et al. 2024, Do et al. 2024a). We used the alignment of the 16S RNA gene sequences to create an ML tree based on a thorough *Anaplasma* and *Ehrlichia* phylogenetic study. The topology illustrated in Figure 4 clearly revealed that, aside from the outgroup *Rickettsia* species (Rickettsiaceae), the phylogenetic tree separated into two well-supported clades, monophyletically positioned for *Anaplasma* and *Ehrlichia* genera, respectively. *Anaplasma platys* isolates from various geographic locations are well grouped into a tight cluster, a sister to the *A. phagocytophilum* subclade. This suggested that the 16S rRNA gene marker, particularly when full length sequences were employed, could clearly distinguish the generic relationships between the two genera. Within each, the species subgroups were well located with a high bootstrap support value (usually greater than 50%), and the taxonomic relationships between *Anaplasma* or *Ehrlichia* taxa were reliably resolved.

Our investigation presented in this study confirms the continuous presence of *A. platys* in domestic dogs and cattle/cows in Vietnam, substantiating previous reports by Parola et al. (2003) and Chien et al. (2019). Furthermore, our findings align with the historical detection of *A. marginale* in dairy cattle (Geurden et al. 2008) and more recent observations by Chien et al. (2019). Notably, the identification of *A. platys* and its dual infection with *A. marginale* reinforces the evidence for the global emergence of this zoonotic rickettsial pathogen, which has been increasingly documented over the past five years (Lima et al. 2020, Hmoon et al. 2021, Kamani et al. 2022, Purisarn et al. 2022, Zobba et al. 2022, Al-Saadi et al. 2023, Ribeiro et al. 2023, Arif et al. 2024, Chadi et al. 2024, Chao et al. 2024, Maas et al. 2024, Bhowmik et al. 2025). This trend highlights *A. platys*' increased importance in cattle infections and its growing potential for zoonotic transmission to humans, raising the same concerns as *A. phagocytophilum* within the Anaplasmataceae family.

CONCLUSIONS

This study confirms the presence of *Anaplasma platys* in family-kept domestic dogs and cattle in Vietnam, highlighting its role as an emerging zoonotic pathogen. The identification of *A. platys* mono-infections in dogs and coinfections with *Anaplasma mafrginale* in cattle — supported by both cytological morulae observation and molecular validation — underscores the complexity of Anaplasmataceae circulation.

Phylogenetic analysis reveals that Vietnamese *A. platys* strains align closely with global isolates, forming a distinct cluster that is sister to *Anaplasma phagocytophilum* in the polyphyletic Anaplasmataceae, with *A. marginale* distantly positioned within the genus.

The presence of this zoonotic pathogen in domestic animals suggests an increased risk of human exposure. Consequently, large-scale surveillance integrating animal hosts, vectors, and human screening is essential to elucidate the epidemiology of anaplasmosis in Vietnam fully.

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Conflict of interest statement.- The authors declare that they have no conflicts of interest.

Credit author statement.- Nguyen Thi Hong Chien – Conceptualization, Funding acquisition, Investigation, Validation, Methodology. Writing – original draft, Project administration. Khue Thi Nguyen – Investigation, Formal analysis, data curation. Thanh Hoa Le – Conceptualization, Data curation, Methodology, Writing, review & editing, Visualization, Supervision, Resources. All authors commented on data analysis, have read and approved the final version of the manuscript.

Data availability statement.- The nucleotide sequences in this study were submitted to GenBank and assigned under the accession numbers: MH686048–MH686050, PX435401–PX435404, and PX485204 for *A. platys*, and MH686043 and PX435405–PX435409 for *A. marginale*.

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Figures

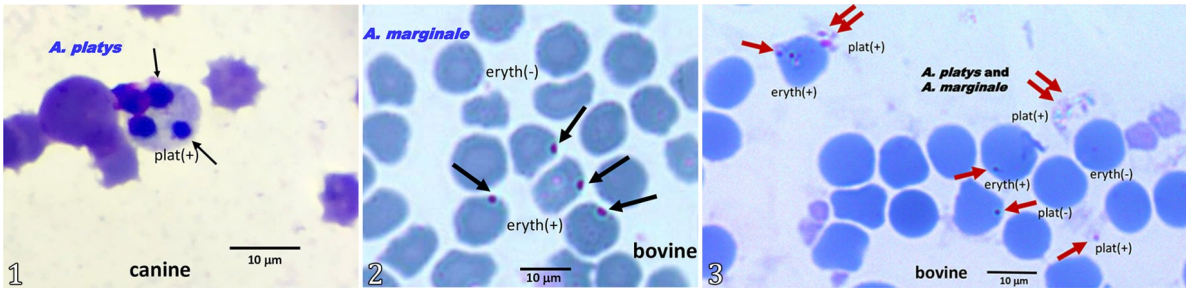

Fig. 1–3. Morulae (indicated by arrows) in peripheral blood smears. (**1**) *Anaplasma platys* forming multiple intracytoplasmic inclusion bodies (morulae) within canine platelets. (**2**) *Anaplasma marginale* morulae located near the membrane of bovine erythrocytes. (**3**) Coinfection in a hybrid dairy cow, showing *A. platys* in platelets and *A. marginale* in erythrocytes. Plat = platelets, eryth = erythrocytes, plat(+) and eryth(+) = infected cells, plat(-) and eryth(-) = uninfected cells. Double arrows = multiple morulae within a single platelet. Diff-Quik stain, obj. 1,000x.

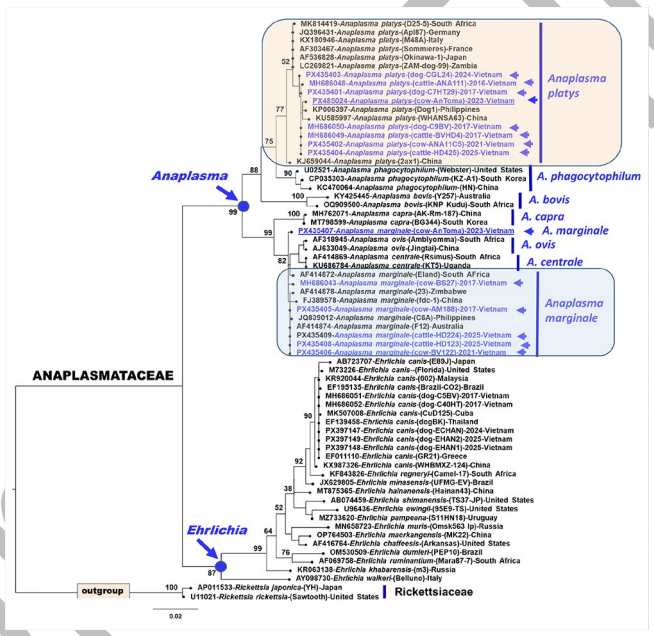

Fig. 4. A maximum-likelihood phylogenetic tree depicting the relationships within and between the *Anaplasma* and *Ehrlichia* genera. The tree was reconstructed based on 67 nucleotide sequences of near full-length 16S rRNA genes from 39 *Anaplasma* strains (seven species), 26 *Ehrlichia* strains (14 species), and two *Rickettsia* species as an outgroup. Sequences were aligned using ClustalW, and the tree was generated in MEGA 11 using the maximum likelihood (ML) method with 1,000 bootstrap replicates (Tamura et al. 2021). The resulting Newick tree was visualized in FigTree v1.4.4. Nodal support values are displayed on each branch. The *Anaplasma* and *Ehrlichia* genera are indicated by solid circles at their respective nodes. Subclades containing *A. platys* and *A. marginale* are enclosed in boxes, with Vietnamese sequences from this study marked by arrows. Isolates from a dually infected cow in Vietnam (PX485024 and PX435407) are underlined. Taxon labels include the accession number, species name, strain designation (in brackets), year of isolation, and country/region of origin. Scale bar = number of substitutions per site.

Table 1. Characteristics of *Anaplasma* species samples from dogs and cattle used for 16S rDNA identification and phylogenetic analysis

No.	Strain designation (Accession No.; Strain; Country)	Year of isolation	Host	Breed sources	Geographical sites	16S rDNA (bp)	Identification *Anaplasma*
1	MH686048-(cattle-ANA111)-2016[a]	2016	*Bos taurus*	Ind.	Ha Noi	1,458	*A. platys*
2	MH686049-(cattle-BVHD4)-2017[a]	2017	*Bos taurus*	Ind.	Ha Noi	1,458	*A. platys*
3	MH686050-(dog-C9BV)-2017[a]	2017	*Canis familiaris*	Ind.	Ha Noi	1,458	*A. platys*
4	PX435401-(dog-C7HT29)-2017	2017	*Canis familiaris*	Ind.	Ha Tinh	1,458	*A. platys*
5	PX435402-(cow-ANA11C5)-2021	2021	*Bos taurus*	Hyb.	Ha Noi	1,458	*A. platys*
6	PX435403-(dog-CGL24)-2024	2024	*Canis familiaris*	Ind.	Ha Noi	1,458	*A. platys*
7	PX435404-(cattle-HD425)-2025	2025	*Bos taurus*	Ind.	Hai Duong	1,458	*A. platys*
8	PX485204-(cow-AnToma)-2023[b]	2023	*Bos taurus*	Hyb.	Ha Noi	1,458	*A. platys*
9	MH686043-(cow-BS27)-2017[a]	2017	*Bos taurus*	Hyb.	Ha Noi	1,482	*A. marginale*
10	PX435405-(cow-AM188)-2017	2017	*Bos taurus*	Hyb.	Hai Duong	1,482	*A. marginale*
11	PX435406-(cow-BV122)-2021	2021	*Bos taurus*	Ind.	Ha Noi	1,482	*A. marginale*
12	PX435407-(cow-AnToma)-2023[b]	2023	*Bos taurus*	Hyb.	Ha Noi	1,482	*A. marginale*
13	PX435408-(cattle-HD123)-2025	3025	*Bos taurus*	Ind.	Hai Duong	1,482	*A. marginale*
14	PX435409-(cattle-HD224)-2025	2025	*Bos taurus*	Ind.	Hai Duong	1,482	*A. marginale*

[a] The 16S rRNA genes of the samples from our previous work (Chien et al. 2019) were sequenced in this study, yielding sequences of 1,458 bp for *A. platys* and 1,482 bp for *A. marginale*; [b] This sample from a cow with a dual infection with *A. platys* and *A. marginale*; Ind. = indigenous, Hyb. = hybrid dairy.

Table 2. The between-group mean genetic distance estimated among the identified species groups of *Anaplasma* inferred from the full-length nucleotide sequences of the 16S rRNA genes

		A. marginale	*A. phagocytophium*	*A. ovis*	*A. bovis*	*A. centrale*	*A. capra*
1	*A. marginale* (n = 11)						
2	*A. phagocytophilum* (n = 3)	0.0355					
3	*A. ovis* (n = 2)	0.0077	0.0382				
4	*A. bovis* (n = 2)	0.0509	0.0371	0.0500			
5	*A. centrale* (n = 2)	0.0079	0.0375	0.0063[a]	0.0489		
6	*A. capra* (n = 2)	0.0191	0.0414	0.0222	0.0570[a]	0.0195	
7	*A. platys* (n = 17)	0.0381	0.0159[b]	0.0399	0.0360	0.0407	0.0430[b]

The mean genetic distance (GD) was calculated using the maximum composite likelihood (MCL), and rate variation between sites was represented by a gamma distribution (shape parameter = 1). All positions with gaps or incomplete data were deleted. The number of base substitutions per site calculated by averaging all sequence pairings between subgroups is shown. [a] Figures with the greatest inter-group distance between *A. capra* and *A. bovis* (5.7%) and the smallest between *A. centrale* and *A. ovis* (0.63%), [b] Lowest value of 1.59% (between *A. platys* and *A. phocytophilum*) and the highest (4.3%) between *A. platys* and *A. capra*.