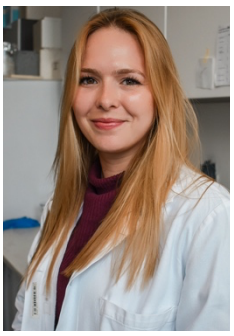
Curriculum vitae



Ana Maria Filipe, Msc

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Nationality: Portuguese
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Current position: PhD candidate at the Laboratory of Molecular Biology of Ticks, Institute of Parasitology, Biology Centre CAS, České Budějovice, Czech Republic
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Research Focus

My research explores the molecular mechanisms that govern the development, transmission and host cell interactions of *Babesia divergens*, an apicomplexan parasite responsible for bovine babesiosis and a life-threatening zoonosis in humans. I focus on key regulatory enzymes, particularly calcium-dependent protein kinases (CDPKs) and clade C aspartic proteases (ASPs), across the parasite's life cycle. One major line of my work involves the characterization of *B. divergens* CDPKs, with particular attention to their stage-specific expression and function during intraerythrocytic development and within infected tick tissues (e.g., salivary glands and ovaries). Using synchronized cultures and a novel tick artificial feeding, I performed multiplex qPCR profiling, revealing differential expression of BdCDPKs and suggesting their roles in processes such as red blood cell invasion, egress, and vector transmission (manuscript in preparation). To probe CDPK function experimentally, I evaluated bumped kinase inhibitors (BKIs) as chemical tools. BKI-1294, originally developed against *Toxoplasma gondii* CDPK1, emerged as the most potent inhibitor of *B. divergens*, inducing a complete block in egress while allowing intracellular replication, resulting in multinucleated complexes. This pharmacological approach enabled functional dissection of egress pathways in the absence of genetic manipulation and further substantiated the role of BdCDPK4 as a key regulator (manuscript in preparation). Complementary to this, I investigated the role of ASPs in red blood cell invasion. I participated on the identification and biochemically characterization of BdASP3a and BdASP3b, homologues of *Plasmodium* PMIX/X, and demonstrated that peptidomimetic inhibitor 49c selectively inhibits BdASP3b, resulting in a severe invasion defect. Using HUNTER proteomics, I mapped 49c-induced disruptions in proteolytic cascades, shedding light on substrate processing events essential for successful host cell invasion (manuscript in preparation). Through a combination of biochemical assays, inhibitor studies, transcriptomics and proteomics, my research aims to define essential molecular players in *Babesia* biology.

Education

2022-now	PhD student, Department of Parasitology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic. Supervisor: Dr. Marie Jalovecká
2020-2021	myClass Advanced C1 course, British Council Lisboa, Lisbon, Portugal
2018-2020	Master student, Department of Animal Biology, Faculty of Sciences, University of Lisbon, Lisbon, Portugal. Supervisors: Dr. David Ramilo and Dr. Maria Teresa Rebelo
2014-2018	Bachelor student, Departments of Animal and Plant Biology, Faculty of Sciences, University of Lisbon, Lisbon, Portugal

Professional Experience

2024-now	Member of the working group led by Dr. Daniel Sojka within the research team at the Laboratory of Molecular Biology of Ticks (headed by Dr. Jan Perner), Institute of Parasitology, Biology Centre CAS, České Budějovice, Czech Republic
2022-2024	Member of the Department of Parasitology (headed by Dr. Eva Nováková), Faculty of Science, University of South Bohemia in České Budějovice, Czech Republic
2022	Member of the Research Unit for Global Infection Control, Department of Global Cooperation (headed by Dr. Masahito Asada), at the National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido, Japan
2018-2020	Member of the Centre for Environmental and Marine Studies, Department of Animal Biology, Faculty of Sciences, University of Lisbon, Lisbon, Portugal

Peer-reviewed publications

Filipe AM., Levytska V., Jalovecká M. (2024) *Babesia divergens*. *Trends in Parasitology* 40, 271-272

Ramilo, D.W., **Filipe, A.M.**, Lucientes, J. et al. (2021) Morphological anomalies found in female *Culicoides* midges (Diptera: Ceratopogonidae). *Biologia* 76, 3405–3410

Attended workshops

2025 Processing and Analysis of Microscopic Images in Biomedicine, Prague, Czech Republic
2024 EMBO Intensive Course in Electron Microscopy for Cell Biology, Oslo, Norway
2024 Sample Preparation Series for Electron Microscopy, Prague, Czech Republic

Conferences

2025 Oral presentation, 4th USB Conference of Doctoral Students, České Budějovice, Czech Republic
2025 Poster presentation, International Proteolysis Society meeting, Búzios, Rio de Janeiro, Brazil
2025 Oral presentation, The 54th Jírovec's Protozoological Days, Frymburk, Czech Republic
2024 Poster and flash talk presentations, EMBL BioMalParXX: Biology and Pathology of the Malaria Parasite, Heidelberg, Germany
2024 Oral presentation, The 53rd Jírovec's Protozoological Days, Karlova Studánka, Czech Republic
2023 Oral presentation, 3rd USB Conference of Doctoral Students, České Budějovice, Czech Republic
2023 Poster presentation, EMBO 13th Young Scientists' Forum Conference, Lisbon, Portugal

Awards and Funding

2025 Best presentation award at the 4th University of South Bohemia (USB) Conference of Doctoral Candidates in České Budějovice, Czech Republic
2025 International Proteolysis Society (IPS) Travel Award for attending IPS meeting in Búzios, Rio de Janeiro, Brazil
2025 Principal Investigator of a one-year student grant project (Grant No. 024/2025/P) titled "Identifying Key Players in the Egress Cascade of *Babesia divergens*"
2024 Registration fee waiver funded by the EMBO Organizer Dr. Norbert Roos for attending the EMBO Intensive Course in Electron Microscopy for Cell Biology
2024 Poster prize winner at EMBL BioMalParXX: Biology and Pathology of the Malaria Parasite, Heidelberg, Germany
2024 Travel grant funded by the EMBL Corporate Partnership Programme for attending the EMBL BioMalParXX: Biology and Pathology of the Malaria Parasite Conference

Certificates and Skills

2023 European Union Aviation Safety Agency A1/A3 subcategory
2020 Certificate of Achievement: Statistics and R - HarvardX
Languages: Portuguese (native), Spanish (intermediate) and English (full professional proficiency, TOEFL ITP certificate)

BSP Spring Meeting 2026, Glasgow, UK - Abstract

Calcium-dependent protein kinase signalling across *Babesia divergens* life cycle

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[1]Faculty of Science, University of South Bohemia in České Budějovice, Czech Republic
[2]Institute of Parasitology, Biology Centre CAS, Czech Republic
[3]Institute for Biology II, University of Freiburg, Freiburg, Germany
[4]Center for Emerging and Re-emerging Infectious Diseases (CERID), Division of Allergy and Infectious Diseases, Department of Medicine, University of Washington, Seattle, Washington, USA
[5]CIBSS - Centre for Integrative Biological Signaling Studies, University of Freiburg, Freiburg, Germany

Babesia divergens is an intraerythrocytic apicomplexan parasite transmitted by *Ixodes ricinus* ticks and a major cause of bovine babesiosis in Europe, with growing recognition as a severe zoonotic pathogen. Parasite survival and transmission rely on tightly regulated processes, including host cell invasion, intracellular replication, egress, and adaptation to both vertebrate and tick hosts. Calcium-dependent protein kinases (CDPKs) are central regulators of these processes in apicomplexan parasites, yet their individual roles in *B. divergens* remain poorly understood.

B. divergens genome encodes four distinct BdCDPK isoenzymes. Stage-specific transcriptional profiling in synchronised intraerythrocytic cultures revealed dynamic expression of each *bdcdpk* associated with developmental transitions, highlighting their potential contributions to parasite biology. BdCDPK4 was recently characterised using inducible knockdown, and the observed phenotype is confirmed here via selective chemical inhibition with bumped kinase inhibitors (BKIs). Inhibition of BdCDPK4 arrested parasite egress without affecting nuclear replication, leading to the accumulation of multinucleated intracellular stages. Comparative phosphoproteomic analysis revealed widespread alterations in phosphorylation patterns, consistent with disruption of a CDPK-centred signalling network. Ultrastructure expansion microscopy confirmed preserved cellular architecture, indicating that the egress block resulted from signalling defects rather than structural damage.

In parallel, conventional knockout of BdCDPK5 demonstrated its essential role in intraerythrocytic replication. Loss of BdCDPK5 function led to complete arrest of parasite reproduction within red blood cells, confirming that BdCDPK5 regulates processes distinct from those controlled by BdCDPK4. To explore functions beyond the blood stage, expression of all four *bdcdpks* was profiled in *I. ricinus* tissues following artificial feeding on infected blood. Tissue-specific expression patterns suggest that individual BdCDPKs contribute to parasite differentiation and transmission within the tick vector, supporting stage- and host-specific kinase regulation. Both BdCDPK4 and BdCDPK5 have been recombinantly expressed and are enzymatically active, enabling ongoing biochemical and structural studies, including three-dimensional structure determination, to guide inhibitor development.

Together, these findings establish BdCDPKs as key regulators of *B. divergens* development across vertebrate and vector hosts. By dissecting the roles of BdCDPK4 and BdCDPK5 and highlighting the broader CDPK signalling network, this work identifies promising targets for interventions aimed at disrupting parasite survival, transmission, and pathogenesis.

Financial disclosure

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