Darwin Fellowship - Final Report

Due within one month of the end date of the Fellowship (maximum 6 pages)

Darwin Fellowship reference	DPlus 198
Name of Darwin Fellow	Katerina Athanasiou
Lead organisation	Hellenic Agricultural Organisation Dimitra
Fellow's organisation(s)	Enalia Physis Environmental Research Centre, Joint Services Health Unit (JSHU) in Sovereign Base Areas Cyprus
Fellow's role within their organisation	• Lab assistant at the JSHU, in SBAs (non-paid position) • Insect surveys and identification (non-paid position) Member of the management committee representing Cyprus for the COST Action CA21170 Pragmatick (non-paid position)
Start/end date of Fellowship	01 April 2023 – 30 March 2025
Location	Sovereign Base Areas of Cyprus (SBAs)
Darwin Fellowship grant value (£)	£49,450
Type of work (e.g. research, training, if other please specify)	Research
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Author(s) and date	Mrs Katerina Athanasiou, Dr Anastasios Saratsis, Dr Kelly Martinou, 29/06/2025

1. Background

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From 2020 to 2023, I served as a volunteer at the Joint Services Health Unit, British Forces Cyprus, located within the Sovereign Base Area of Akrotiri. During this period, I also completed my BSc thesis, which investigated the effects of environmental change on mosquito populations across various habitats of the Akrotiri Peninsula. My academic and professional interests lie in disease vectors affecting both human and animal health, with particular attention to their ecological and biodiversity impacts.

My undergraduate research focused on how land use and environmental quality influence mosquito population dynamics. In addition to my thesis work, I contributed to public

engagement and capacity-building initiatives aimed at increasing awareness of the risks posed by invasive mosquito species and the role of anthropogenic activities in shaping vector ecology. These outreach efforts included educational events for local communities and schools, as well as participation in three international conferences.

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The proposed fellowship seeked to strengthen capacity for the surveillance and monitoring of both established and potentially invasive tick species, along with the pathogens they may carry. The specific aims and objectives of the fellowship were as follows:

- To develop and evaluate tick collection and density estimation methods tailored to the unique environmental and operational conditions of the Sovereign Base Areas (SBAs).
- To conduct systematic sampling for the mapping and identification of tick fauna within the SBAs, including both endemic and invasive species.
- To characterize tick-borne pathogens circulating in the targeted SBAs using molecular diagnostic techniques.
- To assess existing awareness levels and promote knowledge dissemination regarding ticks and tick-borne diseases (TBDs) among relevant stakeholders.

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Dr. Saratsis and Dr. Martinou provided ongoing supervision to Ms. Athanasiou, hosting her at Veterinary Research Institute/ELGO Dimitra (Thessaloniki/Greece) and the Joint Services Health Unit in Cyprus (JSHU), respectively. Dr. Saratsis has provided hands-on training in tick collection techniques, morphological identification, and molecular diagnostic methods. Additionally, he visited the SBAs to monitor and support Ms. Athanasiou's fieldwork activities and participate in a number of events presenting relevant work.

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Ms. Athanasiou received training in tick collection techniques and morphological identification from Dr. Saratsis at the beginning of the project (April-May 2023) and from Dr. Saratsis and Dr. Chitimia-Dobler during the period of 20–23 November 2023. Additionally, she attended a private seminar organized by Dr. Saratsis and featuring Dr. Ben J. Mans, which focused on phylogenetic analysis of genetic sequences. Further training was conducted by Dr. Saratsis in November–December 2024, during which Ms. Athanasiou was instructed in molecular identification of tick species and tick-borne pathogens at the Veterinary Research Institute/ ELGO-Dimitra (Output 1.3/2.3).

2. Achievements

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During the course of the Fellowship, we implemented a structured field survey to monitor tick populations within the Sovereign Base Areas (SBAs). Sampling was conducted every two weeks, from May 2023 to March 2025, utilizing three standardized collection methods: dragging, flagging, and CO_2 traps. Prior to the initiation of repeated samplings, a pilot study was carried out to identify six key areas with considerable tick activity within the SBAs (Output 1.4). Collected specimens were identified to species level using both morphological and molecular techniques. Additionally, ticks were screened for the presence of tick-borne pathogens (TBPs).

As part of our dissemination and capacity-building activities, we participated in several national and international scientific meetings presenting part our results, including:

- The 11th HeLeCos Conference, Patra, Greece (5–7 October 2023)
- ENTO24, Liverpool, United Kingdom (10–12 September 2024)
- The 16th International Symposium on Ticks and Tick-Borne Diseases, Weimar, Germany (26-28 March 2025)

We also contributed to two local events in Cyprus:

- "Monitoring Biodiversity and Zoonoses – The One Health Approach," 13–14 February 2024
- "Drivers of Change: One Health & One Biosecurity," 17–18 February 2025

Moreover, we organized a seminar on ticks and tick-borne diseases on 22 November 2023, hosted at the Akrotiri Environmental Education Centre. The event featured Dr. Anastasios Saratsis and Dr. Lidia Chitimia-Dobler as keynote speakers and was attended by stakeholders, academics, and members of the public.

In the context of COST Action, where I serve as Cyprus's representative on the Management Committee, I attended the MC meeting in Copenhagen, Denmark (18–19 April 2024), as well as a citizen science training school in Novi Sad, Serbia (26–27 September 2024).

In addition to systematic fieldwork, we engaged with local communities by visiting animal owners and veterinarians. Tick specimens were collected from domestic animals, including goats, dogs and cats, particularly in the Limassol district. These samples contributed to a broader understanding of tick biodiversity in the region.

To support and contextualize our fieldwork, a comprehensive literature review was conducted on tick species and tick-borne diseases in Cyprus. Findings revealed that previous studies

predominantly relied on host-based sampling (from domestic or wildlife animals), with no documented use of standardized environmental collection methods as applied in our survey. Most reported cases of tick-borne diseases originated from animals, with only a limited number affecting humans (Output 1.1).

Furthermore, a Knowledge, Attitudes, and Practices (KAP) study was carried out among residents and personnel of both Eastern and Western SBAs. Questionnaires were distributed in English, Greek, and Turkish to assess awareness and behavioral responses concerning tick exposure and disease prevention (Output 1.2).

One logistical challenge encountered was adverse weather conditions, particularly rainfall during the winter months. To address this, field sampling schedules were adjusted, and surveys were conducted on the next available dry days to maintain consistency in data collection. Although not initially planned, we additionally molecularly screened *Ixodes ricinus/inopinatus* complex ticks for *Borrelia* and *Anaplasma/Ehrlichia* spp., given that this species is widely recognized in the literature as a potential vector for these pathogens.

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The primary outcomes of this project include the identification of tick species present in the study area using dragging, flagging, and CO_2 trap collection methods—techniques applied for the first time on the island. Through repeated samplings, three tick species were collected and identified: *Rhipicephalus turanicus* s.l., *Ixodes ventalloi*, and *Ixodes ricinus/inopinatus*. In addition through animal sampling (dogs/cats/sheep/goats) in collaboration with local vets/farmers we were able to additionally collect *R. sanguineus* s.l ticks. Upon phylogenetic analysis of a representative number of the cryptic tick species belonging to the *Ix. ricinus/inopinatus* complex and *Rh. turanicus s.l* group we were able to demonstrate the presence of *Ix. inopinatus* and *Rh. secundus* for the first time in the SBAs and consequently Cyprus. The results of the collected ticks demonstrate clear differences in tick abundance by species and collection method. *Rhipicephalus turanicus s.l.* dominated across all techniques (with an activity peak during spring/summer months), while *I. ricinus/inopinatus* was primarily recovered through flagging (with an activity during winter/spring months). Dragging emerged as the most efficient overall method, reinforcing the need for a multi-technique approach in tick surveillance studies. Species identification was confirmed through both morphological keys and molecular protocols. (Output 2.1, 2.2, 2.3, 2.4).

In addition, the collected ticks were screened using molecular techniques to detect potential pathogens belonging to the genera *Rickettsia*, *Babesia/Theileria*, *Borrelia* and *Anaplasma/Ehrlichia* capable of causing disease in animals and humans (for details on the

sample sizes/methods/results see output 2.3, 2.4 files). Sequencing of PCR products and confirmation of PCR-RLB results followed by BLAST analysis revealed the highest degree of similarity with sequences belonging to *Rickettsia massiliae/rhipicephalii* and *Rickettsia sibirica/parkerii* in *Rh. turanicus s.l.*, *Rickettsia monacensis* in *Ix. inopinatus*, *Ix. ventalloi*, and *Rh. turanicus* s.l., and *Rickettsia helvetica* in *Ix. ventalloi*. In addition, *Rickettsia hoogstraalii* was detected in *H. sulcata* ticks collected outside of the SBAs in Cyprus. Notably, the detection of *R. monacensis* and *R. helvetica* represent novel findings for Cyprus with zoonotic potential. It should be mentioned that both *R. turanicus s.l* and *Ix. inopinatus* ticks are known to be human biting ticks.

PCR analysis targeting *Anaplasma/Ehrlichia* revealed *Midichloria mitochondrii* in *I. inopinatus*, which is a maternally inherited endosymbiont playing a potential role in tick physiology (first report for Cyprus and the SBAs). *Borrelia* spp. infections were not detected in *Ix. inopinatus* ticks. This was also the case for *Babesia/ Theileria* spp. upon screening of 392 ticks belonging to different species.

The Knowledge, Attitudes, and Practices (KAP) study provided insights into community tick identification skills, perceptions and associated practices regarding tick bite prevention and tick-borne diseases. Interestingly, almost 90 % of the 130 participants spent time outdoors at least weekly, and 29 % had experienced a tick bite; of these, 74 % were acquired locally. Approximately 60% of respondents demonstrated positive attitudes towards prevention measures; however, over half reported low levels of actual preventive practices despite awareness of their benefits. Perceived susceptibility, operationalised as the ability to identify at least one high-risk area for tick bites in Cyprus, emerged as the strongest determinant of preventive behaviour (adjusted Odds Ratio = 4.23, 95 % CI 1.78–10.04, p = 0.001). A favourable preventive attitude also remained independently associated with the adoption of preventive measures (adjusted OR = 2.56, 95 % CI 1.14–5.73, p = 0.022)(Output 4.1, 4.2, 4.3).

As part of our citizen science and public outreach efforts, we developed and published educational materials on tick biology and tick-borne diseases via an online platform (https://martinoulab.weebly.com/tick---tick.html). For this purpose, we produced an informational leaflet specifically designed for children, aimed at raising awareness about ticks in an accessible and age-appropriate format. At the onset of the tick season, we also authored a popular science article to inform the general public about tick prevention strategies and measures to protect themselves, their children, and their pets from tick bites [redacted]

(Output 3.1, 3.2, 3.3, 3.4).

3. Outcome, lessons and impact

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  Throughout the Fellowship, I significantly enhanced my skills in tick surveillance and species-level identification, employing both morphological and molecular techniques. These competencies have proven valuable to the Joint Services Health Unit of the British Forces Cyprus, where elements of the tick surveillance protocol have been integrated into their routine monitoring framework. Additionally, I provided training to other personnel on tick collection methodologies and morphological identification at the genus level, contributing to broader capacity-building efforts within the unit.

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  The tick surveillance framework developed during the Fellowship is being integrated into the routine monitoring activities of the Joint Services Health Unit (JSHU). In addition, I am actively involved in training British Forces personnel, including students, in standardized tick collection techniques and morphological identification at the genus level.

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  The Darwin Plus Fellowship has substantially enhanced my ability to tackle real-world biodiversity challenges in my country. One example is the detection of *Ixodes inopinatus* in Cyprus, a finding with significant implications for invasion biology and vector ecology. This tick was found exclusively in a eucalyptus forest within the Sovereign Base Areas, a human introduced habitat originally intended for malaria control and an area that also serves as a key migratory bird corridor. The fellowship supported the development of skills that allowed me to investigate how historical land management and migratory species can intersect to facilitate the establishment of non-native vectors. These insights are crucial for informing future conservation strategies and land-use planning, especially in Mediterranean ecosystems where conflicting environmental goals often coexist.

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  During the Fellowship, I had the opportunity to participate in several scientific conferences, including ENTO24 in Liverpool, United Kingdom, and the COST Action "Prevention, Anticipation, and Mitigation of Tick-Borne Disease Risk Applying the DAMA Protocol (PRAGMATICK)," where I serve as a Management Committee member representing Cyprus. These events provided

valuable opportunities to engage with fellow researchers working in the field of ticks and tick-borne diseases (TBDs), exchange scientific perspectives, and explore potential avenues for future collaboration.

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  N/A

  All supporting documents are provided in the annex, accessible via the following Google Drive link: [redacted]