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# The geographical distribution of the malaria vector *Anopheles arabiensis* in Cabo Verde, 2016–2023

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**Background:** Integrated malaria vector control is crucial to eliminate or reduce infection risk. Understanding vector species distribution, behavior, and environmental factors such as climate, topography, and preferred aquatic habitats is essential.

**Objectives:** Herein, we aim to compile data from 2016 to 2023 on the primary malaria vector in Cabo Verde to support health surveillance and elimination certification.

**Materials and methods:** Mosquitoes larvae were collected using standard sampling tools, and adults using Biogents Sentinel traps, mechanical aspiration and CDC UV light traps, both indoor and in the surroundings of urban and rural areas.

**Results:** Six hundred fifty-seven specimens of *Anopheles gambiae s.l.* were identified by morphological methods and PCR-based techniques as *Anopheles arabiensis* in 12 municipalities from six of the nine inhabited islands, namely, São Vicente, São Nicolau, Maio, Boavista, Santiago, and Santo Antão.

**Conclusions:** We believe this is the first scientific report of the presence of *An. arabiensis* on the island of Santo Antão. The *Anopheles arabiensis* remains the only species in the *An. gambiae* complex in the Cabo Verde islands. These results offer vital insights for epidemiological surveillance and effective malaria control, especially in light of the recent WHO certification declaring the country malaria-free. However, it is imperative to conduct further studies that comprehensively address

epidemiological and entomological aspects, with a special focus on bionomics, genetic determinants of the parasite-vector association, and the characterization of larval habitats. These investigations have the potential to guide a more informed and strategic implementation of malaria vector control measures in Cabo Verde.

KEYWORDS

geographical distribution, *Anopheles arabiensis*, malaria vector control, Cabo Verde, mosquito surveillance

# 1 Introduction

In recent years, an integrated approach to malaria vector control has become increasingly relevant, especially for countries aiming to eliminate or significantly reduce the risk of infection (1). Such evidence-based strategies require detailed knowledge of these vectors' identity, distribution, and bionomics within the target area (2). Recent studies on the spatial distribution of dominant malaria vector species worldwide (3) have begun to address the need for information on the geographical distribution of species, with specific information, including a detailed review of the bionomics (3, 4), thereby allowing disease control programs to improve policy and intervention strategies.

Macro (temperature and topography) and micro (availability of biological niches) factors influence the occurrence of *Anopheles* mosquitoes. This means that in the same region and/or country, geographical complexity, size, different eco-climatic conditions, and topography are significant determinants in the spatial-temporal macro distribution of this primary vector (5–8). The availability of aquatic habitats near human dwellings determines the occurrence of mosquito vectors in the communities (9, 10). Dispersal of adult mosquitoes is typically seasonal and follows rainfall patterns, resulting in different population densities between and within countries (9). Vector control is critical in eliminating malaria in endemic areas (11, 12). On January 12th, 2024, Cabo Verde was officially certified by the WHO as a malaria-free country after six consecutive years without local cases and more than 20 years of efforts to reach such a significant goal (13). The last record of a malaria epidemic in the country was in 2017 (14), with 446 total cases, 423 of which were local and centered mainly in Praia. Therefore, intervention measures were readapted, focusing on eliminating the disease, and since January 2018, the country has not reported any local cases of the disease (15).

Certification on malaria elimination is the official recognition by WHO of a country's malaria-free status. The certification is granted when a country has shown – with rigorous and credible evidence – that the chain of indigenous malaria transmission by *Anopheles* mosquitoes has been interrupted nationwide for at least the past three consecutive years. A country must also demonstrate the capacity to prevent the re-establishment of transmission (1). Despite the target of zero local malaria, a robust surveillance system in the country, which includes entomological surveillance, becomes essential to keep the country free of the disease and prevent reintroduction. Significant gains have been made in several areas. However, entomological studies on the distribution and bionomics of the primary malaria vector, *Anopheles arabiensis* Patton, in the country remains a major challenge.

A recent study on mosquito fauna updates in Cabo Verde has provided comprehensive information on the distribution of mosquito species in the country. Data compilation and surveys spanned all nine islands and 22 municipalities, identifying ten mosquito species, including four vectors of human pathogens: *An. arabiensis*, *Aedes aegypti* Linnaeus, *Culex pipiens* (Linnaeus) senso stricto (s.s.), and *Culex quinquefasciatus* Say (16). These findings are crucial to bolster the country's entomological surveillance system, ensuring the sustainability of malaria elimination efforts, preventing potential reintroductions, and guarding against the introduction or resurgence of other vector-borne diseases.

Despite the achieved certification, the country still faces challenges related to imported cases of malaria, stemming from international trade and tourism activities between endemic regions. In this scenario, maintaining robust surveillance becomes crucial to prevent the reintroduction of transmission after the importation of malaria cases. Thus, a comprehensive understanding of the geographical distribution of the *An. arabiensis* mosquito emerges as a fundamental element to solidify the success in preventing the reintroduction of the disease, thereby ensuring the durability of the malaria-free status.

With the aim of providing relevant information and substantial data to reinforce the entomological surveillance system, this study focused on compiling data regarding the geographical distribution of the malaria vector mosquito, *An. arabiensis*, in Cabo Verde from 2016 to 2023. This compilation initiative is part of the ongoing entomological surveillance activities conducted by the Medical Entomology Laboratory of the National Institute of Public Health in collaboration with the National Malaria Control Program and other national and international entities.

# 2 Materials and methods

## 2.1 Study sites

Located 500 km off the west coast of Africa, Cabo Verde is an archipelago of ten islands, nine of which are inhabited. The country has 22 municipalities at the administrative level, where the decentralized organs of the National Health System are located. With a population of about 491,233 inhabitants (17), the fragmentation of its territory creates challenges at several levels, especially related to preventive health responses. The islands are located below the tropic of Cancer and have a semi-desert tropical climate tempered by the trade winds, which blow constantly. Temperatures vary little throughout the seasons, with the maximum between 25°C and 30°C from August to October, while the minimum temperatures range between 19°C and 25°C from December to March. The hottest period of the year aligns with the water season, occurring in August and September, the hottest and wettest months, respectively, with temperatures exceeding 20 °C. The archipelago's climate and precipitation levels are influenced by the Intertropical Convergence Zone (ITCZ), resulting in an average annual rainfall of 197 mm (18), which is sufficient to maintain the mosquito population throughout the year despite the low volume of rainfall concentrated in a single season.

## 2.2 Mosquito collection

Under the entomological surveillance activities led by the National Institute of Public Health, mosquito collections were made in areas randomly selected in the 22 municipalities from 2016 to 2023. During the study, all water sources considered potential breeding sites were inspected, recording information about the type of breeding site, its location, presence of vegetation, sunlight exposure, and the presence of larvae of other mosquito species.

The breeding sites were classified as temporary, retaining water for a limited period, and permanent, maintaining water throughout the year. Temporary sites included water drums, water tanks plant saucers, and tires, while permanent sites comprised streamlets.

### 2.2.1 Collection of larvae and pupae

The collection of immature mosquitoes (larvae and pupae) was performed by inspection of immatures indoors and in urban and rural areas. All larvae and pupae collected in the different municipalities were transported to the National Institute of Public Health Medical Entomology Laboratory for identification.

### 2.2.2 Collection of adult mosquitoes:

For the collection of adult mosquitoes, various techniques were employed, including the use of Biogents Sentinel traps (with BG-Lure and $CO_2$ baits), mechanical aspiration using Procopack and backpack aspirators, as well as the use of CDC light traps with ultraviolet light. Subsequently, the adult mosquitoes collected were sent to the Medical Entomology Laboratory of the National Institute of Public Health of Cabo Verde for the identification process.

## 2.3 Mosquito species identification

Mosquito specimens (larvae and adults) were identified morphologically using identification keys (19, 20). All *An. gambiae s.l.* larvae were preserved in 70% ethanol and adults in silica gel (Merck KGaA, Darmstadt, Germany) at room temperature or dry at -20°C for posterior DNA extraction. Each specimen was subjected to total genomic DNA extraction using the NZY Tissue DNA isolation kit (NZYTech genes & enzymes, Lisbon, Portugal). Identification of cryptic species of the *An. gambiae* complex was performed by PCR amplification of ribosomal DNA (rDNA) following the protocol described by Scott et al. (21). For molecular identification, a universal primer was used for the species complex (5'-GTGTGCCCCTTCCTCGATGT-3') and specific primers for *An. arabiensis* (5'-AAGTGTCCTTCTCCATCCTA-3'), *An. gambiae s.s.* (5' CTGGTTTGGTCGGCACGTT-3') and *An. melas/merus* (5'-TGACCAACCCACTCCCTTGA-3').

## 2.4 Ethics statement

All mosquito collections were made in private (residences and vicinities) and public spaces, after obtaining oral consent from the owner. The field collections did not involve any threatened or protected species. The procedures and materials used in the collections did not pose any health risk to researchers or owners and did not involve any vertebrate animals.

# 3 Results

During the study, all sources of water considered potential breeding sites were inspected. A total of 402 larvae of *An. arabiensis* were collected. Remarkably, *An. arabiensis* larvae were predominantly found in outdoor and temporary habitats, such as tanks and water drums exposed to sunlight, often used in agriculture. Additionally, larvae were observed in permanent habitats, such as small streams with clean water and some vegetation. In all inspected habitats where *An. arabiensis* larvae were present, the presence of larvae from other mosquito species, including *An. pretoriensis* (Theobald,1903) and culicid larvae, specifically *Cx. pipiens* s.l. and *Ae. aegypti* (*Linnaeus, 1762*), was also recorded, suggesting frequent habitat sharing among different mosquito species (Table 1). The permanent breeding sites with the presence of *An. arabiensis* larvae were the usual ones, showing a higher percentage of larvae after the short rainy season. The low number of these breeding sites may be associated with the short rainy season, vector control, and urbanization. For the collection of adult *An. arabienis* mosquitoes, a variety of techniques were employed, encompassing the utilization of Biogents Sentinel traps baited with BG-Lure and $CO_2$ (obtained from a mixture of yeast, sugar, and water), mechanical aspiration via Procopack and backpack aspirators, and the deployment of CDC light traps with ultraviolet light. A total of 255 adult *An. arabienis* mosquitoes were captured. Among the adult samples, 177 were females, of which

TABLE 1 Characteristics of *Anopheles arabiensis* breeding sites..

| Island | Municipality | Year collected | Breeding sites | Location | Water characteristics | Water permanence | Vegetation | Sunlight exposure | Association with other species |
|---|---|---|---|---|---|---|---|---|---|
| Santo Antão | Porto Novo | 2022 | Water tanks | Outdoor | Cloudy | Temporary | Absent | Exposed | Yes |
| São Vicente | São Vicente | 2017–2022 | Water drums | Outdoor | Clear | Temporary | Absent | Exposed | Yes |
| | | | Water tanks | Outdoor | Cloudy | Temporary | Absent | Exposed | Yes |
| São Nicolau | Ribeira Brava | 2022 | Streams | Outdoor | Clear | Permanent | Present | Exposed | Yes |
| | | | Water drums | Outdoor | Clear | Temporary | Absent | Exposed | Yes |
| Boavista | Sal Rei | 2016–2022 | Water tanks | Outdoor | Clear | Temporary | Absent | Exposed | Yes |
| | | | Buckets | Outdoor | Clear | Temporary | Absent | Exposed | Yes |
| | | | Puddles | Outdoor | Clear | Temporary | Absent | Exposed | Yes |
| | | | Others | Outdoor | Polluted | Permanent | Present | Exposed | Yes |
| Santiago | Santa Catarina | 2016–2022 | Water drums | Outdoor | Cloudy | Temporary | Absent | Exposed | Yes |
| | | | Animal fountains | Outdoor | Cloudy | Temporary | Present | Exposed | Yes |
| | | | Others | Outdoor | Cloudy | Permanente | Present | Exposed | Yes |
| | | | Streamlets | Outdoor | Cloudy | Permanent | Absent | Exposed | Yes |
| | São Lourenço dos Órgãos | 2016–2022 | Water tanks | Outdoor | Clear | Temporary | Present | Exposed | Yes |
| | | | Water tanks | Outdoor | Clear | Temporary | Absent | Exposed | Yes |
| | | | Water drums | Outdoor | Clear | Temporary | Absent | Exposed | Yes |
| | | | Water drums | Outdoor | Clear | Temporary | Absent | Exposed | Yes |
| | São Salvador do Mundo | 2016–2022 | Water drums | Outdoor | Cloudy | Temporary | Absent | Exposed | Yes |
| | | | Water tanks | Outdoor | Cloudy | Temporary | Present | Exposed | Yes |
| | Ribeira Grande | 2017–2022 | Water drums | Outdoor | Clear | Temporary | Present | Exposed | Yes |
| | | | Water tanks | Outdoor | Cloudy | Temporary | Present | Exposed | Yes |
| | Praia | 2016–2022 | Plant saucers | Indoor | Clear | Temporary | Present | Not Exposed | Yes |
| | | | Streamlets | Outdoor | Clear | Permnanent | Absent | Exposed | Yes |
| | | | Water tanks | Outdoor | Clear | Temporary | Absent | Exposed | Yes |
| | | | Streamlets | Outdoor | Clear | Permanent | Absent | Exposed | Yes |
| | | | Water tanks | Outdoor | Clear | Temporary | Absent | Exposed | Yes |

*(Continued)*





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TABLE 1 Continued

| Island | Municipality | Year collected | Breeding sites | Location | Water characteristics | Water permanence | Vegetation | Sunlight exposure | Association with other species |
|---|---|---|---|---|---|---|---|---|---|
| Maio | Maio | 2017 | Water tanks | Outdoor | Clear | Temporary | Present | Exposed | Yes |
| | | | Animal fountains | Outdoor | Cloudy | Temporary | Absent | Exposed | Yes |

only 14 were pregnant and 11 had recently fed, while 83 were males. The traps were deployed on the field for one week.

For the identification of *Anopheles gambiae* complex species, a total of 657 samples of *An. gambiae* s.l. collected from 2016 to 2023 were submitted to PCR for species confirmation, of which 99.5% (n=654) were successfully amplified and identified as *An. arabiensis* and 0.5% (n=3) did not amplify.

The *An. arabiensis* mosquitoes identified during the study period were found in 12 municipalities distributed across six Cabo Verde islands, as shown in Figure 1.

## 4 Discussion

Understanding the geographical -distribution and management of *An. arabiensis* larval breeding sites is essential for malaria surveillance. In our study, the species was initially identified on Santo Antão Island, within the Porto Novo municipality, particularly in Tarrafal de Monte Trigo. On São Nicolau Island, it was observed within the Ribeira Brava municipality, specifically in Caleijão and Água das Patas. *An. arabiensis* has been consistently identified on Santiago Island since 2016, spanning all nine municipalities: Praia, São Domingos, São Lourenço dos Órgãos, São Salvador do Mundo, Tarrafal, Santa Catarina, Santa Cruz, São Miguel, and Ribeira Grande. São Vicente saw the species' identified in both 2017 and 2022, while on Maio Island it was found in 2017 and on Boavista Island, both in 2017 and 2022. Each of these islands boasts a single municipality bearing its name (Figure 1).

In the study, *An. arabiensis* larval breeding sites were predominantly temporary, attributed to the need for water storage due to irregular water distribution in some areas. Due to vector control interventions, permanent breeding sites are scarce, well-known, and georeferenced, with low larval density (22). The short duration of these breeding sites is attributed to the low frequency of rainfall in the country. It was observed that most of these breeding sites consisted of small collections of clean water exposed to sunlight, primarily tanks, linked to the need for water storage. Despite being predominantly located outside dwellings, proximity to residential areas may increase people's exposure to mosquito bites. Therefore, effectively identifying and managing these breeding sites are crucial to reducing the risk of mosquito-borne disease transmission.

Furthermore, *An. arabiensis* larvae were consistently found in association with other mosquito species, such as *An. pretoriensis, Ae. aegypti,* and *Cx. pipiens* s.l. This coexistence is explained by the predominance of these other species in the country (23), together with the characteristic climate and vegetation of a dry tropical environment with short periods of rainfall, which do not favor the creation of many suitable breeding sites for mosquitoes. The scarcity of suitable breeding sites leads to the sharing of these locations, resulting in competition for survival among species, contrasting with the dynamics observed in some countries in the African sub-region, such as Senegal (24), Ghana (25), and The Gambia (26).

Identifying mosquito species, mapping their geographical distribution and other aspects of their bionomics are essential to

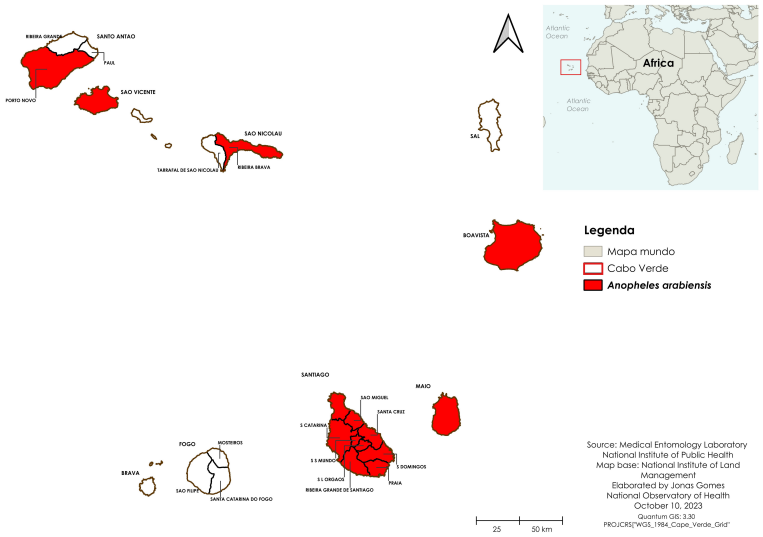


FIGURE 1
Geographical distribution of *An. arabiensis* species in Cabo Verde municipalities, from 2016 to 2023. Municipalities with *Anopheles arabiensis:* 1. Porto Novo; 2.São Vicente; 3.Tarrafal; 4. Boavista; 5. Maio; 6. Praia; 7.Ribeira Grande de Santiago 8.São Domingos; 9. São Lourenço dos Órgãos; 10. São Salvador do Mundo; 11. Santa Cruz;.12. Santa Catarina; 13. São Miguel; 14. Tarrafal de Santiago.

identify risk areas and implement targeted vector control strategies. This information is particularly important in the process of certification and maintaining of a malaria-free country.

The *An. gambiae* species complex contains sub-Saharan Africa's most important critical malaria vector mosquitoes. It comprises eight cryptic species, and the main malaria vectors in the complex are *An. gambiae* sensu stricto (s.s.), *An. arabiensis* and *An. coluzzii* (27, 28). From our data, with the analysis of molecular markers, we confirm, once again, the presence of the species *An. arabiensis* in the archipelago as reported in other studies (29–33). If on one side, Alves et al. (20, 30) reported the presence of the vector on all the islands except Santo Antão and São Vicente, the compilation of the WHO case study, 2012 (31), defends the presence of the vector on all the islands, except for Sal and Brava. However, this latter study (31), does not present any data or reference to any methodology used in the field studies and the identification of the species used.

Despite the presence of *An. arabiensis* in six (66.7%) of the inhabited islands, the species density seems to be low because of the recent extended periods of drought that have kept the number of breeding sites low. This first finding of the species *An. arabiensis* in Santo Antão island and again in São Nicolau, compared to the studies of Alves et al. (30), although with less frequency, may be related to the increased mobility of goods and people to and from these islands, or simply to the increase/improvement of entomological surveillance activities in recent years.

The development and tourist exchange between the Cabo Verde islands and the countries where malaria is endemic may regularly favor the importation of malaria cases into the islands, representing a greater risk for the islands that have the vector and that have had autochthonous cases of the disease, namely, Santiago and Boavista (32). This threat of a resurgence of malaria (because of the presence of the malaria vector and imported cases) is a significant public health problem. This calls for strengthened planning to prepare for eventual resurgence of malaria and for a comprehensive long-term approach to prevent mosquito-associated diseases.

Although in Cabo Verde this species is known to be associated with the transmission of *Plasmodium falciparum* in the past, the agent responsible for the autochthonous malaria cases in the country, recent data showed that this mosquito species is weakly anthropophilic, which may partly explain the low number of malaria cases in the country (33). In conclusion, *An. arabiensis* remains the only species of the *An. gambiae* complex in the islands of Cape Verde. The absence of malaria in islands where *An. arabiensis* is present, but without reported autochthonous cases, underscores the need for more in-depth investigations. These studies should focus on epidemiological and entomological dynamics, particularly in bionomics, and genetic influences in the parasite-vector interaction. The characterization of larval habitats provides valuable insights, not only consolidating the exclusive role of *An. arabiensis* as a malaria vector in Cabo Verde but also providing a solid foundation to guide effective vector control strategies. The incorporation of these data into public health policy-making is crucial to maintain the malaria-free status of the country, highlighting the epidemiological importance of these findings in the broader context of vector-borne disease control.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

## Author contributions

SL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. CS: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing – review & editing. DM: Data curation, Formal analysis, Investigation, Writing – review & editing. MM: Formal analysis, Funding acquisition, Writing – review & editing. AG: Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. ADP: Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note



## Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fitd.2024.1353839/full#supplementary-material

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