Molecular detection and maternal transmission of a bacterial symbiont *Asaia* species in field-caught *Anopheles* mosquitoes from Cameroon

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**Abstract**

**Background:** Malaria control relies mainly on insecticide-based tools. However, the effectiveness of these tools is threatened by widespread insecticide resistance in malaria vectors, highlighting the need for alternative control approaches. The endosymbiont *Asaia* has emerged as a promising candidate for paratransgenic control of malaria, but its biology and genetics still need to be further analyzed across Africa. Here, we investigated the prevalence of *Asaia* and its maternal transmission in the natural population of *Anopheles* mosquitoes in Cameroon.

**Methods:** Indoor-resting adult mosquitoes belonging to four species (*An. coluzzii*, *An. arabiensis*, *An. funestus* and *An. gambiae*) were collected from eight localities across Cameroon from July 2016 to February 2020. PCR was performed on the *Asaia*-specific 16S ribosomal RNA gene, and samples positive by PCR for *Asaia* were confirmed by Sanger sequencing and phylogenetic analysis. The vertical transmission of *Asaia* was investigated by screening *F*1 mosquitoes belonging to *F*0 *Asaia*-positive females.

**Results:** A total of 895 mosquitoes were screened. We found 43% (384) *Asaia* infection prevalence in four mosquito species. Phylogenetic analysis revealed that *Asaia* from Cameroon clustered together with the strains of *Asaia* isolated from other parts of the world. In addition, seven nucleotide sequence variants were found with low genetic diversity (*π* = 0.00241) and nucleotide sequence variant diversity (Hd = 0.481). *Asaia* was vertically transmitted with high frequency (range from 42.5 to 100%).

**Conclusions:** This study provides field-based evidence of the presence of *Asaia* in *Anopheles* mosquitoes in Cameroon for exploitation as a symbiont in the control of malaria in sub-Saharan Africa.

**Keywords:** Malaria, *Anopheles*, *Asaia*, Genetic diversity, Maternal transmission, *Plasmodium* detection, Cameroon

**Background**

Malaria is considered one of the most devastating diseases in sub-Saharan Africa. The World Health Organization (WHO) World malaria report 2019 recorded 216 million cases of malaria and 409,000 deaths worldwide in 2019 [1]. In Cameroon, malaria is endemic, with the whole population being at risk [2].
Stable natural infection of Asaia in Anopheles mosquitoes has been shown in different species of Anopheles around the world [16, 17, 26] and more recently in African malaria vectors, notably An. gambiae, An. funestus and An. coluzzii [19, 27]. To our knowledge, only one report of Asaia has been documented in An. coluzzii and An. gambiae from Yaoundé, Cameroon, which focused on its detection by 454 pyrosequencing [28]. There is a paucity of available data on the prevalence of Asaia in other Anopheles species and in other localities across the country. In addition, the genetic diversity and the stability of the maternal transmission of Asaia in Anopheles mosquito are yet to be assessed in the Cameroonian context.

Methods

Study sites and sample collection
Mosquitoes were collected in eight localities in Cameroon, namely Gounougou (Northern Region 9°03′00″ N, 13°43′59″ E), Tibati (Adamaoua Region, 6°28′00″ N, 12°38′00″ E), Mibellon (Adamaoua Region, 6°46′ N, 11°70′ E), Mangoum (West Region, 5°28′00″ N, 12°22′30″ E), Elon (Centre Region, 4°15′00″ N, 11°37′00″ E), Elende (Centre Region, 3°41′57.27″ N, 11°33′28.46″ E) and Obout (Centre Region, 3°28′17.00″ N, 11°44′09.4″ E) (Fig. 1), from July 2016 to February 2020. The samples from Mibellon, Gounougou and Bankeng were collected in the rainy season, while those from Elende, Elon, Obout, Mangoum and Tibati were collected in the dry season. Indoor resting female mosquitoes were collected using electric aspirators between 06:00 a.m. and 09:00 a.m. following verbal consent from the chief and each household representative. The collected mosquitoes were then transported to the insectary at the Centre for Research in Infectious Diseases (CRID), Yaoundé, where they were morphologically identified following morphological identification keys for Afrotropical anopheline mosquitoes [29].

DNA extraction and molecular species identification
Total genomic DNA (gDNA) of each adult mosquito was extracted using the Livak method [30]. Following extraction, the concentration and purity of the extracted gDNA were determined using a NanoDrop™ spectrophotometer.
Fig. 1  Map of the sampling sites. The study sites where the samples were collected are represented by stars. The map was constructed for this publication in QGIS 3.14 (https://www.qgis.org/fr/site/index.html) using country and regional boundaries from GADM (https://gadm.org/download_country_v3.html)
were then tested with their respective FAM-based polymerase chain reaction (PCR) [31] and cocktail PCR [32] were performed to identify the different species of the An. gambiae complex and An. funestus group, respectively.

### Plasmodium infection rates

The detection of Plasmodium infection from each whole mosquito was performed using the TaqMan assay as described previously [33]. In this assay, two probes were used to check for the presence or absence of Plasmodium infection. The first probe, labeled with FAM, detects P. vivax, P. ovale and/or P. malariae. This indicates that the effect of bacteria on Plasmodium development has been based only on the presence versus absence of Plasmodium infection.

### Asaia screening and sequencing

The presence of Asaia in the mosquitoes was detected with a diagnostic PCR using Asaia-specific primers 16SF (5′-TGG CGG ACG GG TGT GTT CAT C-3′) and Asarev (5′-AGC GTC AGT AAT GAG CCA GTG T-3′) [26] to amplify 676 base pairs (bp) of the 16S rRNA gene. The reaction mix was run for 5 min at 95 °C and cycled 35 times through 30 s at 94 °C, 30 s at 60 °C and 1 min at 72 °C. Finally, further extension was performed for 10 min at 72 °C. PCR products were then resolved and visualized on 1.5% agarose gel containing Midori green dye. If bands of the expected size were visible on the gels, the PCR products were cleaned up using Exonuclease I (Exo I) and Shrimp Alkaline Phosphatase (Exo-SAP protocol) according to the New England Biolabs protocol (NEB, Ipswich, MA, USA) and sent for sequencing. Sterile water was used as a negative control.

### Assessing the vertical transmission of Asaia

To assess the vertical transmission of Asaia in Anopheles mosquitoes, we investigated the presence of the bacteria in matching F₀ female parent and F₁ female An. funestus progeny from Elende. Blood-fed adult mosquitoes collected in Elende were transferred individually to paper cups and allowed to feed for 4–5 days on 10% sterile sugar-soaked cotton wool. Once fully gravid, females were allowed to oviposit individually by forced egg laying as described previously [34]. After hatching, larvae were reared to obtain F₁ adults in isofamilies. F₀ mosquitoes were then tested with their respective F₁ for the presence of Asaia. Detection for the presence of Asaia by PCR in F₁ mosquitoes was also performed, and the positive samples were sequenced to assess whether the Asaia strains from F₀ to F₁ individuals were the same. The Asaia sequences from F₁ progeny were aligned and compared with the sequences of their respective F₀ parents. This was done to confirm the vertical transmission pattern of Asaia.

### Phylogenetic analysis

Overall, 60 individual samples were analyzed using the 16S rRNA gene. The sample distribution included 10 An. funestus samples each from Mibellon, Obout, Elon and Elende, 10 An. gambiae samples from Mibellon, four An. gambiae samples from Bankeng, five An. coluzzii samples from Gounougou, and one An. funestus sample from Tibati. The number of samples utilized was based on the number of infected mosquitoes among the various species and the clarity of the sequences. Maximum likelihood phylogenetic trees were constructed using the Asaia-specific 16S rRNA gene target. The taxonomic relationships of the strains obtained from this study were inferred against GenBank sequence isolates. These existing isolates are presented in Additional file 1: Table S1. Additionally, these sequences were aligned with the ClustalW multiple sequence alignment tool in BioEdit software. Neoasaia chiangmaitensis (FJ887939.1), Gluconobacter oxydans (KU255083.1) and Acetobacter tropicalis (JF930138.1) were used as outgroups.

The evolutionary history was inferred by the maximum likelihood method using MEGA X based on the Jukes–Cantor model. The robustness of the individual branches was estimated by bootstrapping with 1000 replicates. All the sequences were deposited in GenBank under the accession numbers MW450601–MW450660. Genetic parameters including the number of nucleotide sequence variants, nucleotide sequence variant diversity (Hd), Tajima’s and Fu’s indexes and nucleotide diversity were computed using DnaSP 5.10.01. The TCS program was used to construct the haplotype network [35].

### Statistical analysis

Data were computed in MS Excel and analyzed using R software version 1.1.463. The Chi-square test was used to compare the prevalence of Asaia between different localities and species. The comparison of the prevalence of P. falciparum in Asaia-positive and Asaia-negative individuals was done using Fisher’s exact test.

### Results

#### Prevalence of natural Asaia species and infection rates in Anopheles mosquitoes

Overall, 895 Anopheles mosquitoes belonging to four species, namely An. funestus, An. gambiae, An. arabiensis and An. coluzzii, were screened to determine the prevalence of Asaia sp. The results showed a general prevalence of Asaia sp. up to 43% (95% CI = 40–50%) (Table 1).
respectively. An. gambiae populations, 56.4% and Elon and Elende. In the prevalence was 100% in from Obout, registered in rate of Asaia Anopheles different localities and of was 51.6%, compared to 2.2% from Tibati. The prevalence

\[ \chi^2 \] 

An. gambiae \[ \chi^2 = 47.2, df = 1, P = 0.001 \]. On the other hand, the prevalence of Asaia in An. gambiae from Mibellon was comparable to that of An. gambiae from Bankeng \( \chi^2 = 0.47, df = 1, P = 0.490 \).

According to mosquito collection seasons, out of the 458 An. funestus mosquitoes tested, 336 were collected in the dry season and 122 in the rainy season (Table 3). The prevalence of Asaia during the dry (51.6%) and rainy (47.3%) seasons was the same for An. funestus \( \chi^2 = 0.67, df = 1, P = 0.413 \), showing that season does not have an impact. Regarding An. gambiae, 131 mosquitoes were collected during the rainy season, with an Asaia infection rate of 58.8%, while only 48 were collected during the dry season, with no infection observed (Table 3). This suggests that the rainy season has a positive effect on the prevalence of Asaia in An. gambiae \( \chi^2 = 47.14, df = 1, P = 0.0001 \).

**Phylogenetic analysis and genetic diversity of Asaia sp.**

The 470-bp sequences obtained were subjected to BLAST search in GenBank (NCBI), and all had homology with Asaia sp. The relationship between Asaia sp. and Anopheles mosquitoes, notably An. funestus, An. gambiae and An. coluzzii, revealed a clustering of the same species of Asaia irrespective of the locality or species (Fig. 2a). Comparison of the sequences obtained to the reference revealed close relatedness of Asaia strains in five out of the 10 samples (50%) of An. funestus mosquitoes from Mibellon, and all the An. funestus (100%) from Elende, Elon and Obout. The strains shared close similarity with Asaia strains A. platycodi, A. prunellae, A. siamensis and A. lannensis isolated from An. gambiae from Senegal [21], Asaia strain AE isolated from Ae. aegypti from Italy [23] and other Asaia strains isolated from An. stephensi from Italy [17].

### Table 1 Infection rates of Asaia according to Anopheles species

| Species          | Tested | Infected | Infection rate (%) | 95% Confidence interval |
|------------------|--------|----------|--------------------|-------------------------|
| An. coluzzii     | 243    | 82       | 34                 | 28–40                   |
| An. arabiensis   | 15     | 3        | 20                 | 4–48                    |
| An. funestus     | 458    | 222      | 48.5               | 44–53                   |
| An. gambiae      | 179    | 77       | 43                 | 36–51                   |
| Total            | 895    | 384      | 43                 | 40–50                   |

All the species of Anopheles studied were found to be infected by Asaia, with infection rates of 43%, 48.4%, 33.7% and 20% for An. gambiae, An. funestus, An. coluzzii and An. arabiensis, respectively (Table 2).

The prevalence of Asaia varied according to mosquitoes species. Indeed, Asaia prevalence was significantly higher in An. funestus than in An. coluzzii (\( \chi^2 = 3.39, df = 1, P = 0.00028 \)) but was similar to the infection rate of An. gambiae (\( \chi^2 = 1.33, df = 1, P > 0.05 \)) and An. arabiensis (\( \chi^2 = 3.64, df = 1, P = 0.142 \)). Likewise, the prevalence of Asaia in An. gambiae did not differ from that in An. coluzzii (33.7%) \( \chi^2 = 3.39, df = 1, P = 0.066 \) or An. arabiensis (\( \chi^2 = 2.15, df = 1, P = 0.154 \)). Focusing on the different localities and Anopheles species, the infection rate of Asaia registered in An. funestus from Mibellon was 51.6%, compared to 2.2% from Tibati. The prevalence of Asaia was 0% in An. gambiae from Mangoum, whereas the prevalence was 100% in An. funestus from Obout, Elon and Elende. In An. gambiae populations, 56.4% and 64.9% were Asaia-positive in Mibellon and Bankeng, respectively. Anopheles funestus mosquitoes from Mibellon were found to be more frequently infected by Asaia

### Table 2 Infection rate of Asaia in Anopheles mosquitoes according to species and locality

| Localities | Species          | Tested | Infected | Infection rate (%) | 95% Confidence interval |
|------------|------------------|--------|----------|--------------------|-------------------------|
| Mibellon   | An. funestus     | 122    | 63       | 51.6               | 42–61                   |
|            | An. gambiae      | 94     | 53       | 56.4               | 46–67                   |
| Tibati     | An. funestus     | 181    | 4        | 2.2                | 0.6–5                   |
|            | An. gambiae      | 71     | 71       | 100                | 95–100                  |
| Obout      | An. funestus     | 71     | 71       | 100                | 95–100                  |
|            | An. gambiae      | 48     | 0        | 0                  | 0–7                     |
| Mangoum    | An. gambiae      | 48     | 43       | 100                | 92–100                  |
|            | An. arabiensis   | 15     | 15       | 100                | 91–100                  |
| Elon       | An. funestus     | 43     | 43       | 100                | 92–100                  |
|            | An. gambiae      | 242    | 242      | 100                | 92–100                  |
| Gounougou  | An. coluzzii     | 81     | 81       | 100                | 88–90                   |
|            | An. arabiensis   | 15     | 3        | 20                 | 4–48                    |
| Bankeng    | An. gambiae      | 37     | 37       | 64.9               | 47–80                   |
|            | An. coluzzii     | 1      | 1        | 0                  | 2.5–100                 |

| Seasons  | Species          | Tested | Infected | Infection rate (%) | 95% Confidence interval |
|----------|------------------|--------|----------|--------------------|-------------------------|
| Dry season | An. funestus      | 316    | 159      | 50                 | 45–56                   |
|           | An. gambiae      | 48     | 0        | 0                  | 0–7                     |
| Rainy season | An. funestus    | 122    | 63       | 51.6               | 42–61                   |
|           | An. gambiae      | 131    | 77       | 58.8               | 50–67                   |
|           | An. coluzzii     | 243    | 82       | 33.7               | 28–40                   |
|           | An. arabiensis   | 15     | 3        | 20                 | 4–50                    |
| Total    |                  | 895    | 384      | 43                 | 40–50                   |
Similarly, five out of 10 (50%) An. funestus mosquitoes and one out of the four (25%) An. gambiae from Bankeng were found to be infected by a strain of Asaia sp. that clusters with A. krungthepensis and Asaia strain AGF0 of An. gambiae from Burkina Faso [18]. Moreover, Asaia sp. found in the 10 An. gambiae mosquitoes from Mibellon, one An. coluzzii (20%, 1/5) from Gounougou and An. funestus from Tibati cluster with A. astilbis.

Genetic diversity parameters of Asaia sp. revealed seven distinct nucleotide sequence variants (Fig. 2b), and low genetic diversity ($\pi=0.00241$) and nucleotide sequence variant diversity ($Hd=0.481$) (Table 4). The result also showed six variable polymorphic sites (Fig. 2c) with slight nucleotide differences. Depending on the mosquito species, we recorded nucleotide diversity of 0.00128, 0.00278 and 0.00084 for An. funestus, An. gambiae and An. coluzzii, respectively (Table 4). The major nucleotide sequence variant H1 ($n=42$, frequency $=70\%$) was present in three species of mosquitoes originating from four locations (Mibellon, Elende, Bankeng and Gounougou). This suggests that H1 could be the original nucleotide sequence variant. H3 ($n=11$, frequency $=18.3\%$) was found in An. gambiae from Mibellon and An. funestus from Tibati cluster with A. astilbis.

The nucleotide sequence variant H4 ($n=1$, frequency $=1.6\%$) was detected in An. coluzzii from Gounougou. H5 ($n=1$, frequency $=1.6\%$), H6 ($n=1$, frequency $=1.6\%$) and H7 ($n=1$, frequency $=1.6\%$) nucleotide sequence variants were present exclusively in An. funestus from Mibellon (Fig. 2b). The overall Tajima’s $D$
belonging to 29 isofamilies. We found that 601 mosquitoes used to determine the proximity to neutrality were negative and non-significant for the *Asaia* strains circulating in the *Anopheles* population from the different localities (Table 4).

*Asaia* sp. is vertically transmitted and displays stability of infection in *Anopheles* mosquitoes

Although *Asaia* is mainly maternally transmitted, horizontal transmission may occasionally occur in natural conditions. To confirm the vertical transmission in the infected mosquito species, we analyzed the infection status of 651 *An. funestus* $F_1$ mosquitoes from Elende belonging to 29 isofamilies. We found that 601 $F_1$ mosquitoes were *Asaia*-positive, with average vertical transmission frequency of 91.5% (range: 42.5–100%) (Fig. 3). In addition to the high transmission of *Asaia* in $F_1$ mosquitoes, comparative phylogenetic analysis of the *Asaia* sequences from $F_0$ to their $F_1$ progeny revealed identical clustering (Fig. 4). Moreover, this prevalence of transmission could include both vertical and horizontal transmission because the internal control of testing progeny of *Asaia*-negative parents in order to exclude horizontal transmission through laboratory rearing was lacking in this study.

*Asaia* has no effect on *Plasmodium* infection in *An. funestus* from Mibellon

We compared the overall *P. falciparum* infection rates (based on the presence vs. absence of the *Plasmodium* infection) in *An. funestus* mosquitoes collected from Mibellon to determine a possible correlation with the presence of *Asaia* infection. Of the 122 mosquitoes sampled, only 25% had detectable *Asaia*-*Plasmodium* co-infections, as opposed to 51% *Asaia* mono-infections. Furthermore, only 31 out of the 122 (26%) mosquitoes were *Plasmodium*-positive. There was no association between the prevalence of *Plasmodium* in *Asaia*-negative and *Asaia*-positive groups ($\chi^2=1.9, df=1, P>0.05$) of *An. funestus* from Mibellon. Thus, *Asaia* has no effect on the natural *Plasmodium* infection status of *Anopheles* mosquitoes in Mibellon (Fig. 5).

**Table 4** Genetic parameters

| Locality  | Species      | $n$ | $S$ | $h$ | $Hd$ | $\pi$     | Tajima’s $D$ | Fu’s $F_s$ | FuLiD | FuLiF |
|-----------|--------------|-----|-----|-----|------|----------|-------------|------------|-------|-------|
| Gounougou | *An. coluzzi*| 5   | 1   | 2   | 0.40 | 0.00084 | $-0.81650$  | $0.090$    | $-0.81650$ | $-0.77152$ |
| Mibellon  | *An. funestus* | 10  | 4   | 4   | 0.7333 | 0.00377 | 1.04827   | 0.361      | 1.23914   | 1.33492 |
|           | *An. gambiae* | 10  | 0   | 1   | –    | 0.00000 | –          | 0          | 0.00000   | 0.00000 |
| Obout     | *An. funestus* | 10  | 0   | 1   | –    | 0.00000 | –          | 0          | 0.00000   | 0.00000 |
| Elende     | *An. funestus* | 10  | 0   | 1   | –    | 0.00000 | –          | 0          | 0.00000   | 0.00000 |
| Elon      | *An. funestus* | 10  | 0   | 1   | –    | 0.00000 | –          | 0          | 0.00000   | 0.00000 |
| Bankeng   | *An. gambiae* | 4   | 3   | 2   | 0.5  | 0.00319 | $-0.75445$ | 1.716      | $-0.75445$ | $-0.67466$ |
| Pooled    | *An. funestus* | 40  | 4   | 4   | 0.235 | 0.00128 | $-0.85441$ | $-0.555$   | 1.02694   | 0.50379 |
|           | *An. gambiae* | 14  | 5   | 3   | 0.473 | 0.00278 | $-0.58260$ | 1.456      | $-0.95239$ | $-0.88944$ |
| Total     | –            | 60  | 6   | 7   | 0.481 | 0.00241 | $-0.29815$ | $-1.335$   | 1.16049   | 0.81346 |

Discussion

This study provides an estimate of the prevalence of *Asaia* in natural populations of *Anopheles* mosquitoes across different localities of Cameroon. The mosquito species studied here have an overall *Asaia* prevalence of 43%. This prevalence is lower than the prevalence (70%) reported in *Anopheles* mosquitoes in Iran [26]. Likewise, it is also lower than the prevalence documented in the Centre Region of Cameroon, where *Asaia* frequency of 95% was observed in the midguts of *Anopheles* mosquitoes [28]. This difference could be explained by the seasonal, geographical and environmental variation in microbiota composition in *Anopheles* mosquitoes from the various study sites [36–38].

We also found that all the *Anopheles* species were infected by *Asaia*. This correlates with previous studies in the Centre Region of Cameroon where *Asaia* infection was recorded in *An. gambiae* and *An. coluzzii* mosquitoes [28]. This finding also parallels that of a study in Madagascar that demonstrated *Asaia* infection in *An. funestus* mosquitoes [27]. The presence of natural *Asaia* in several *Anopheles* species could be harnessed to develop a paratransgenic control method against *Anopheles* vectors responsible for malaria. The fact that *Asaia* has also been described in other species of mosquitoes such as *An. stephensi* [17] and *Ae. aegypti* [23] suggests that *Asaia* is not restricted to certain mosquito species. Rather, it can be widely distributed among many vector species. Moreover, *Asaia* has been reported to be stably associated with different mosquito species owing to its vertical and horizontal (through pre-adult and adult
mosquito oral feeding) transmission potential. This also accounts for its dominance in the mosquito microbiome [17, 18, 23]. Furthermore, Asaia can be found in different niches, including mosquito breeding sites and also in flower nectars. These ecological segments constitute a suitable habitat for these bacteria [21].

The results obtained show variability in infection rates between localities in different species of Anopheles. This is explained by the heterogeneous routes in transmission including feeding on flowers, breeding site, and horizontal and vertical dissemination events [17, 21, 22]. The highest infection rates of Asaia were observed at Elon, Elende and Obout, which are all located in the forest region. This highest infection rate could be attributed to the abundance of plants and breeding sites. Indeed, it has been demonstrated that flower nectar is home to viable strains of Asaia [21].

The clustering of the Asaia strains obtained in this study with other known species of Asaia suggests that the strains of Asaia infecting Anopheles mosquitoes in Cameroon are not novel species. Further investigations are needed to identify each species of Asaia circulating in Anopheles populations across Cameroon. Moreover, the close relatedness with the Asaia strain isolated from other mosquito species from other countries suggests that introducing genetically modified Asaia strains in the Anopheles population will override genetic hurdles of mosquito populations isolated by reproductive barriers. These reproductive barriers often occur in endemic malaria settings and account for limitations observed with vector control strategies. In addition, the overall negative values for both neutrality tests (Tajima’s D and Fu’s Fs tests) based on the 16S rRNA gene indicate an excess of the rare mutations in populations, which suggest a recent population expansion as already demonstrated in a previous study focusing on the genetic diversity of Echinococcus granulosus complex using mitochondrial DNA [39]. Also, analysis of the 16S rRNA gene confirmed that all the strains belong to the genus Asaia, but it was not possible to identify the species, and further analyses such as whole-genome sequencing could be used to better characterize Asaia strains of Anopheles mosquitoes in Cameroon.

This study demonstrated that Asaia-infected females transmit the infection with high frequency (91.5%) to their progeny, suggesting a vertical transmission of Asaia.
Moreover, the close relatedness of the *Asaia* strains between *F*<sub>1</sub> and *F*<sub>0</sub> female mosquitoes suggests that *Asaia* displays stability of infection and reinforces the vertical transmission potential of the bacteria in *Anopheles* mosquitoes. This feature is crucial because it offers the possibility for introducing engineered *Asaia* into mosquito populations in the field which will spread over time and replace the wild-type population. The vertical transmission of *Asaia* has also been shown in other species of *Anopheles* [13, 17]. Our results suggest that *Asaia* is a promising candidate for bacterial engineering for the production of anti-plasmodium effectors. In fact, the high vertical transmission frequency of *Asaia* is not a surprising event, as it has already been demonstrated in *Anopheles* mosquitoes [17, 18]. Nevertheless, the high frequency of *Asaia* transmission recorded in this study could be due to a combination of horizontal and vertical dissemination events during co-feeding of *F*<sub>1</sub> mosquitoes in the laboratory. Given that the presence of *Asaia* has not been tested in *Asaia*-negative parents, we cannot exclude the possibility that the DNA sequences detected in this study came from some sort of environmental contamination through sugar meals or other sources. Additional experiments would be of great interest to demonstrate actual infection, for example, showing maternal transmission of the bacteria from *Asaia*-negative samples and intracellular localization of the sequences. Also, different techniques such as fluorescence in situ hybridization (FISH) could be used to detect *Asaia* in different tissues (head and thorax, midgut and ovaries). Finally, the estimated rate of transfer of *Asaia* vertically from parents to progeny reported here also includes the rate at which *F*<sub>1</sub> larvae acquired infection from laboratory sources.

Several distinct microbial-based approaches are currently being investigated [9–11]. However, it is unclear whether these approaches are compatible. For example, there is increasing evidence that microbe–microbe interactions influence colonization and abundance within the host [40–42], and these parameters are likely critical for successful control strategies. This is true for *Asaia* and *Wolbachia*, which appear antagonistic to one another [43, 44]. The recent finding of stable *Wolbachia* infection in *Anopheles* mosquitoes reinvigorates the use of this microbe for control of malaria [27, 45]. As such, there need to be careful considerations for the type of microbial control approach to be used in a particular region given that it may impede future control strategies.

*Asaia* has been shown to be a promising candidate for paratransgenic control of malaria. This is as a result of its negative effect on the development of *Plasmodium* at different stages [27]. Here we focused on the effect of *Asaia* on *Plasmodium* in natural populations. The data obtained show that in a field *Anopheles* population of Mibellon, the presence of *Asaia* did not affect the infection of mosquitoes by *Plasmodium*. These conclusions are in accord with the results of a recent study demonstrating that *Asaia* and *Wolbachia* do not influence *Plasmodium* infection in natural populations [27]. However, Bassene et al. showed that a strain of *Asaia* correlated negatively

![Fig. 4 Phylogenetic tree showing the relationship between *Asaia* strains isolated in *F*<sub>0</sub> and *F*<sub>1</sub> mosquitoes. *Ele* *F*<sub>0</sub> represents the *F*<sub>0</sub> female *Asaia*-positive sample collected at Elende, and *Ele* *F*<sub>1</sub> the respective progeny from Elende. Hap 1–6 represent the other haplotypes found in this study.](attachment://fig4.png)

![Fig. 5 Correlation between *Asaia* and *Plasmodium* at Mibellon. The comparison of the prevalence of *Plasmodium* in *Asaia*-positive and *Asaia*-negative *An. funestus* mosquitoes at Mibellon was done using Fisher’s exact test. *P* +: *Plasmodium*-positive; *P* −: *Plasmodium*-negative](attachment://fig5.png)
with *Plasmodium* infection in wild populations of *An. gambiae* sensu lato and *An. funestus* in Senegal [19]. Moreover, experimental infection studies need to be conducted to better evaluate the relationship between *Asaia* and *Plasmodium* infection in wild *Anopheles* mosquitoes. It will also be important to quantify the level of *Plasmodium* parasites and *Asaia* sp. in each *Anopheles* population to better determine this correlation.

**Conclusion**

*Asaia* has been presented as a promising candidate for alternative control of malaria. Our study provides preliminary evidence of the circulation of *Asaia* in malaria vectors in Cameroon. The predominance of a nucleotide sequence variant in all the mosquito species suggests the feasibility of a paratransgenic control approach via the bioengineering of *Asaia* in malaria vectors. These data provide important initial baseline information towards developing potential strategies by exploring the possibility of utilizing this strategy for malaria control.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s13071-021-05044-2.

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**Authors’ contributions**

MMS and CSW conceived the study. MMS designed the study. CGM, MMS, ANK, MT and BM collected the samples in the field. CGM and MMS performed the genetic analysis. MMS and CSW wrote the manuscript with contributions from MOK, GLH, CSW and FN. All authors read and approved the final manuscript.

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**Availability of data and materials**

The data are part of a wider study of bacteria detection in *Anopheles* mosquitoes in Cameroon. Data are available from the corresponding author upon reasonable request.

**Declarations**

**Ethics approval and consent to participate**

Verbal community acceptance was obtained from the village local council representatives. Oral consent for sampling inside houses was obtained from household owners before collection.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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