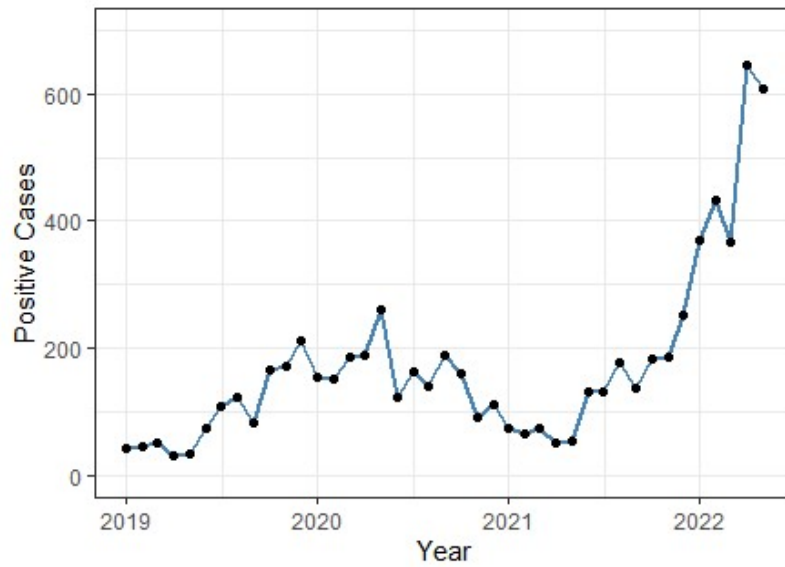


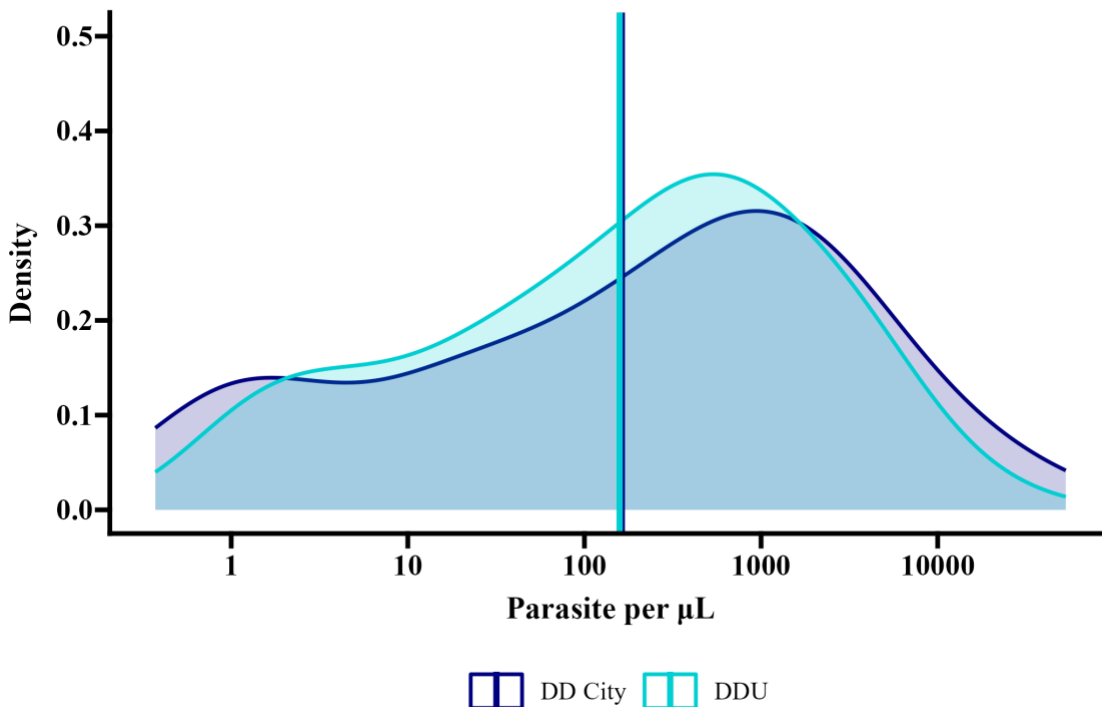
# Evidence for a role of *Anopheles stephensi* in the spread of drug- and diagnosis-resistant malaria in Africa

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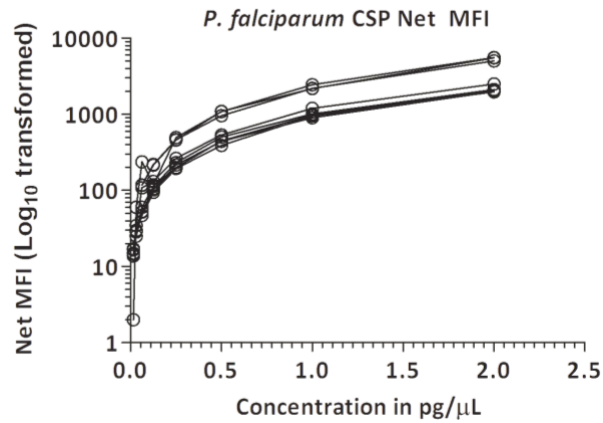
In the format provided by the  
authors and unedited



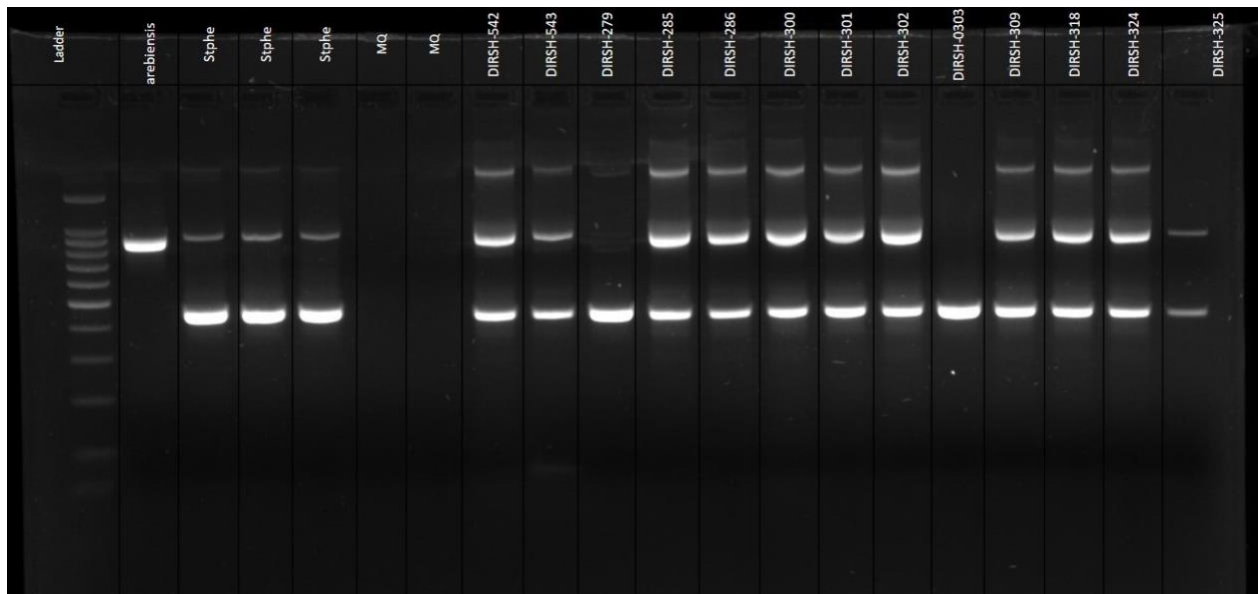
**Figure S1: Number of monthly malaria positive cases in Dire Dawa city from January 2019 to May 2022.** Data obtained from DHIS2 and collected from the 34 health facilities is only up to May 2022.



**Figure S2: Parasite density distribution between university students and city residents with relative averages.**



**Figure S3: Summary of the standard curves generated using recombinant proteins specific for *P. falciparum*.**



**Figure S4: Example gel picture of ITS2 based PCR for confirmation of *An. stephensi*.** In these assays, colony *An. arabiensis* (Lane 1) and *An. stephensi* (Lane 2-4) mosquitoes were included as positive extraction and PCR controls and MQ (Lane 5-6) as a negative PCR control together with wild caught morphologically *An. stephensi* identified mosquitoes (Lanes 7-19). The first lane to the left is a 100bp molecular weight marker (labelled Ladder). The experiment outcomes were consistent with morphological outcomes. We did not repeat the PCR experiments for species identification.

**Table S1: Net Mean Fluorescence Intensity (MFI) values of recombinant proteins and positive control mosquitoes**

| Recombinant protein                                                                                                              | Concentration<br>in pg/ $\mu$ L | Net MFI of First Batch Experiments |          |          |          |          |
|----------------------------------------------------------------------------------------------------------------------------------|---------------------------------|------------------------------------|----------|----------|----------|----------|
|                                                                                                                                  |                                 | Exp't 1                            | Exp't 2  | Exp't 3  | Exp't 4  | Exp't 5  |
| <i>P. falciparum</i> CSP<br>recombinant protein 2-<br>fold serial dilution used<br>for the validation of CSP<br>bead-based assay | 2                               | 1,975.8                            | 2,498.5  | 2,110.5  | 2,073.0  | 1,982.5  |
|                                                                                                                                  | 1                               | 948.8                              | 1,202.0  | 1,008.5  | 970.5    | 904.0    |
|                                                                                                                                  | 0.5                             | 390.8                              | 533.0    | 503.0    | 447.5    | 441.5    |
|                                                                                                                                  | 0.25                            | 193.8                              | 265.0    | 232.5    | 200.0    | 216.0    |
|                                                                                                                                  | 0.125                           | 94.3                               | 131.0    | 112.5    | 102.0    | 105.5    |
|                                                                                                                                  | 0.0625                          | 46.8                               | 108.0    | 60.0     | 52.0     | 52.5     |
|                                                                                                                                  | 0.03125                         | 29.8                               | 34.5     | 29.5     | 25.0     | 28.5     |
|                                                                                                                                  | 0.015625                        | 13.8                               | 17.0     | 16.5     | 15.0     | 14.5     |
| Positive colony mosquitoes used as<br>extraction and CSP quality control                                                         |                                 | 281.0                              | 306.0    | 24,376.5 | 24,560.0 | 24,043.5 |
|                                                                                                                                  |                                 | 19,314.0                           | 19,005.0 | 21,203.5 | 24,234.0 | 26,086.5 |

MFI, mean fluorescence intensity; Exp't #, experiment number; N/A, not available; CSP, circumsporozoite protein

43 **Table S2: Net Mean Fluorescence Intensity (MFI) values of negative adult *An. stephensi* mosquitoes**  
 44 **reared from larvae used as a negative control**

| Exp't #   | ID                  | Pf |
|-----------|---------------------|----|
| Exp't #01 | NC-01               | 46 |
| Exp't #01 | NC-02               | 47 |
| Exp't #01 | NC-03               | 48 |
| Exp't #01 | NC-04               | 49 |
| Exp't #02 | NC-01               | 50 |
| Exp't #02 | NC-02               | 51 |
| Exp't #02 | NC-03               | 52 |
| Exp't #02 | NC-04               | 54 |
| Exp't #03 | NC-05               | 55 |
| Exp't #03 | NC-05               | 56 |
| Exp't #03 | NC-05               | 57 |
| Exp't #04 | NC-05               | 58 |
| Exp't #04 | NC-04               | 59 |
| Exp't #04 | NC-04               | 60 |
| Exp't #04 | NC-04               | 61 |
| Exp't #05 | NC                  | 62 |
| Exp't #05 | NC                  | 63 |
| Exp't #05 | NC                  | 64 |
| Exp't #05 | NC                  | 65 |
| Exp't #05 | NC                  | 66 |
| Exp't #05 | NC                  | 67 |
| Exp't #05 | NC                  | 68 |
| Exp't #05 | NC                  | 69 |
| Exp't #05 | NC                  | 70 |
| Exp't #05 | NC                  | 71 |
| Exp't #05 | NC                  | 72 |
| Exp't #05 | NC                  | 73 |
| Exp't #05 | NC                  | 74 |
| Exp't #05 | NC                  | 75 |
| Exp't #06 | NC (DDL-232)        | 76 |
| Exp't #06 | NC (DDL-499         | 77 |
| Exp't #06 | NC (DDL-232)        | 78 |
| Exp't #06 | NC (DDL-233)        | 79 |
| Exp't #06 | NC (DDUIC-025 #6-2) | 80 |
|           | average             | 81 |
|           | SD                  | 82 |
|           | average + 3*SD      | 83 |

85 NC, negative control; Exp't #, experiment number; SD, standard deviation; Pf, *P. falciparum*

86 **Table S3: Variable selection results.**

|                                                                                         | Full data                                                                                                           | City                                                                                            | University                                                                                                  |
|-----------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|
| <b>Screened variables (<math>p</math> value &lt;0.3).</b>                               | Site, larvaepos, larvaespp, larvaesteph, adultspindoor, adultspoutdoor, dist_river, SEX, REP, IRS, LLIUSE, stephpos | Stephpos, latitude, adultspindoor, adultspoutdoor, dist_river, SEX, AGE, REP, LLINLN, WT12, EDU | Stephpos, longitude, larvaepos, larvaespp, adultspindoor, adultspoutdoor, travelprop, AGE, EAVE, LLIH, WT12 |
| <b>Number of models tested</b>                                                          | 4096                                                                                                                | 2048                                                                                            | 2048                                                                                                        |
| <b>Min AIC</b>                                                                          | 431                                                                                                                 | 213                                                                                             | 198                                                                                                         |
| <b>Max AIC</b>                                                                          | 598                                                                                                                 | 260                                                                                             | 210                                                                                                         |
| <b>Variables in the top 10% of models (Contributing to at least 20% of the top 10%)</b> |                                                                                                                     |                                                                                                 |                                                                                                             |
|                                                                                         | Distance river (83%)                                                                                                | Distance river (98%)                                                                            | Longitude (97%)                                                                                             |
|                                                                                         | Site (45%)                                                                                                          | Stephensi positive (49%)                                                                        | Larvae positive (64%)                                                                                       |
|                                                                                         | Sex (27%)                                                                                                           | Sex (48%)                                                                                       | Stephensi positive (44%)                                                                                    |
|                                                                                         | Larvaepos (25%)                                                                                                     | Education (20%)                                                                                 | Larvae Stephensi (25%)                                                                                      |
|                                                                                         | Adult spp outdoor (22%)                                                                                             | Adult spp outdoor (20%)                                                                         |                                                                                                             |
|                                                                                         | Larvae Stephensi (20%)                                                                                              |                                                                                                 |                                                                                                             |

87  
88 **Table S4: Results from a multi-level logistic regression model**

| Factors                                           | Category         | Fitted models |         |               |         |               |         |
|---------------------------------------------------|------------------|---------------|---------|---------------|---------|---------------|---------|
|                                                   |                  | Model 1       |         | Model 2       |         | Model 3       |         |
|                                                   |                  | Estimate (SE) | P value | Estimate (SE) | P value | Estimate (SE) | P value |
| Sex                                               | Female (Ref.)    |               |         |               |         |               |         |
|                                                   | Male             | 1.1 (0.3)     | <0.001  | 0.6 (0.3)     | 0.044   | 0.6 (0.3)     | 0.034   |
| Age in years                                      | < 5 years (Ref.) |               |         |               |         |               |         |
|                                                   | 5 -15 Years      | 1.3 (0.7)     | 0.071   | 0.4 (0.6)     | 0.509   | 0.6 (0.6)     | 0.358   |
|                                                   | Above 15 Years   | 1.5 (0.7)     | 0.029   | 0.8 (0.5)     | 0.126   | 1.0 (0.6)     | 0.063   |
| <i>An. stephensi</i> larvae and/or adult presence | Absent (Ref.)    |               |         |               |         |               |         |
|                                                   | Present          | 1.2 (0.4)     | <0.001  | 1.6 (0.4)     | <0.001  | 1.5 (0.4)     | <0.001  |
| Natural waterbody presence                        | Absent (Ref.)    |               |         |               |         |               |         |
|                                                   | Present          | 0.6 (0.3)     | 0.089   | 0.4 (0.4)     | 0.241   | 0.5 (0.4)     | 0.149   |
| Usage of aerosol insecticide spray                | Not Using (Ref.) |               |         |               |         |               |         |
|                                                   | Using            | -1.3 (0.5)    | 0.016   | -1.6 (0.6)    | 0.005   | -1.4 (0.5)    | 0.007   |
| <b>Fit Statistics</b>                             |                  |               |         |               |         |               |         |
| -2log(likelihood)                                 |                  | 755.2         |         | 859.8         |         | 833.8         |         |
| AIC (smaller is better)                           |                  | 775.4         |         | 879.9         |         | 853.9         |         |
| BIC (smaller is better)                           |                  | 823.3         |         | 927.7         |         | 901.7         |         |

89 **Estimated regression coefficients (SE),  $p$ -value from Wald test and fit statistics for the multi-level**  
90 **logistic regression model.**