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Building the vector in? Construction practices contribute to the invasion and persistence of *Anopheles stephensi* in Jigjiga, Ethiopia

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Abstract

Anopheles stephensi is a major vector of malaria in Asia and the Arabian Peninsula, and its recent invasion into Africa poses a significant threat to malaria control and elimination efforts on the continent. The mosquito is well-adapted to urban environments, and its presence in Africa could potentially lead to an increase in malaria transmission in cities. Most of the knowledge about *An. stephensi* ecology in Africa has been generated from studies conducted during the rainy season, when vectors are most abundant. Here, we provide evidence from the peak of the dry season in the city of Jigjiga, Ethiopia, and report the finding of *An. stephensi* immature stages infesting predominantly water reservoirs made to support construction operations (in construction sites or associated with brick manufacturing businesses). Political and economic changes in Ethiopia (and particularly the Somali Region) have fueled an unprecedented construction boom since 2018 that, in our opinion, has been instrumental in the establishment, persistence and propagation of *An. stephensi* via the year-round availability of perennial larval habitats associated with construction. We argue that larval source management during the dry season may provide a unique opportunity for focused control of *An. stephensi* in Jigjiga and similar areas.

Introduction

Remarkable success in reducing malaria burden has been achieved in most African countries since the year 2000, thanks to the scaling-up of vector control tools (insecticide-treated nets and indoor residual spraying) and effective preventive and treatment drugs[1]. Increasing evidence suggests that rapid urbanization of Africa's human population (driven primarily by rural-urban immigration) is also contributing to a reduction in malaria burden [2-5]. Lower habitat suitability for *Anopheles spp.* breeding and improvements in housing within African cities reduce human-mosquito contacts and can lead to lower *Plasmodium* spp. inoculation rates compared to rural settings [2-5]. Environmental management in the form of housing improvement has gained research interest due to its sustained effect on *Anopheles* spp. mosquitoes and its positive impact on livelihoods [6,7]. The WHO calls this approach "building the vector out", and involves the adoption of practices that range from improved housing structures to retrofitting eave tubes and other approaches to limit mosquito entry indoors [8]. This approach is also seen as a novel aspect of malaria control in urban settings, given most human population growth over the next century will be accounted for by the growing number of city dwellers [9].

As most sub-Saharan countries continue their push towards malaria elimination, a new threat has the potential to negatively impact decades of public health gains: the invasion and establishment of *Anopheles stephensi*, a malaria vector native to Asia, commonly found in cities throughout India, Iran, Pakistan, and the Arabian Peninsula [10,11]. Since it was first detected in Africa in Djibouti in 2012[12], *An. stephensi* has spread to Ethiopia[13], Somalia[14], Sudan[15], Kenya, Nigeria and Ghana[16]. Niche modeling predicts suitable environmental conditions for *An. stephensi* establishment throughout tropical African cities, putting an additional 126 million people potentially at risk of malaria [17]. Given the mosquito's urban dependency and container larval breeding habits [18,19], rainfall alone was found to be a poor predictor of *An. stephensi*-driven malaria transmission [20]. In Djibouti, a 2000x exponential increase in the number of malaria cases has been observed since the detection of *An. stephensi* [11,16]. The contribution of *An. stephensi* to increases in malaria transmission outside of Djibouti has begun to be investigated, especially in light of the recent malaria outbreak. From 2018 to 2020 in Ethiopia, *Plasmodium vivax* was detected in wild-caught *An. stephensi* from the cities of Dire Dawa and Kebridehar (with infection rates of 0·5% and 0·3%, respectively)[19] and *P. vivax* and *P. falciparum* infection recently reported in *An. stephensi* from Awash (2·8% and 1·4%)[21]. Furthermore, experimental membrane feeding experiments showed that field-caught *An. stephensi* from Ethiopia became significantly more infectious with local *P. vivax* and *P. falciparum* than *Anopheles arabiensis* (the primary malaria vector in Ethiopia), indicating that it is a highly competent vector for African *Plasmodium*[21].

Given the entomological and epidemiological evidence gathered so far, the World Health Organization (WHO) launched a new initiative to stop the further spread of *An. stephensi* in the region that is based on a 5-pronged approach: 1. increasing

91 collaboration; 2. strengthening surveillance; 3. improving information exchange; 4.
92 developing guidance; and 5. prioritizing research[16]. To execute an effective plan for *An.*
93 *stephensi* elimination, key sources of information about its biology and bionomics in its
94 new habitats are needed, and vector control tools that are better suited for urban
95 settings will need to be investigated.

96 Several studies (most of them from Ethiopia, cross-sectional and conducted during the
97 rainy season) have characterized *An. stephensi* habitats and bionomics, with many
98 knowledge gaps still remaining (e.g., [18,19,21,22]). The evidence gathered so far shows that
99 in the rainy season, *An. stephensi* larvae are found in a wide array of small and large
100 artificial containers, ranging from large water cisterns to car tires and buckets [18,19,21]. In
101 addition to *Plasmodium* infection, such studies have characterized up to 48% human
102 biting (14/29 mosquitoes) in Awash[21] but low human biting (<1% human biting) in Dire
103 Dawa and Kebridehar (where also a high frequency of domestic animal feeding was
104 observed)[19]. Such discrepancies may have originated, in part, due to the opportunistic
105 collection of adult mosquitoes in or near animal shelters. Indeed, the finding of *P. vivax*
106 and *P. falciparum* infected mosquitoes can only be explained by human biting.
107 Furthermore, given its egg-laying behavior (eggs that resist desiccation [23] and are laid in
108 small containers), the fact that it bites humans not only at night when they are sleeping,
109 and that it is found in urban and peri-urban areas, *An. stephensi* has more similarities
110 with *Aedes aegypti* mosquitoes (which vector viruses such as dengue, chikungunya and
111 Zika) than with other Anophelines [24]. One of the many factors that remains to be studied
112 is how is it that *An. stephensi* persists in Ethiopia and other African countries that have
113 a prolonged dry season, as this period may offer unique opportunities for surveillance
114 and control.

115 Here, we report novel findings on the habitat use of *An. stephensi* during the dry season
116 in eastern Ethiopia. While increased focus on characterizing larval habitats in rainy
117 periods can provide information of niche breadth for the species, our goal of focusing on
118 the dry season was to explore possible windows for control in periods where the
119 population size may be smallest.

120 Dry season *An. stephensi* collections in Jigjiga, Ethiopia. From March 6-14, 2023,
121 mosquito surveys were conducted in Jigjiga city (capital of Somali Region, Ethiopia,
122 population ~800,000) during the dry season. *Anopheles stephensi* was first detected in
123 Jigjiga in 2018[18], and has persisted in the city since then despite a harsh dry season
124 (the rainless period of the year lasts for ~3 months). Molecular analysis of cytochrome
125 oxidase subunit I (*COI*) and cytochrome B gene (*CytB*) shows Jigjiga as one of the
126 locations with highest diversity, suggesting it was likely an early introduction point of *An.*
127 *stephensi* into Ethiopia[25]. Jigjiga is of relevance because of its large population size,
128 rapid urbanization, and connection to other malaria-endemic regions and the port of
129 Berbera in Somaliland.

130 We employed methods developed for standard larval and pupal sampling of container
131 breeding mosquitoes[8], that included collecting all the larvae and pupae in small water

132 holding containers and using dippers and large fish nets to sample large water-holding
133 habitats. All the larvae and pupae were reared to Adult at Jigjiga University Entomology
134 Laboratory. The emerged *Anopheles* spp. adults were identified to species level using
135 standard keys as well as molecular means. From a total of 60 potential larval sites with
136 water that were sampled across the city, we identified a major habitat consistently
137 positive for *An. stephensi* larvae and pupae during the dry season: man-made pits
138 related with construction operations (Fig 1A). We term such habitats 'construction pits',
139 as they were primarily built for the storage of water in construction sites or in small-scale
140 brick manufacturing businesses (Fig 1B). *Anopheles stephensi* positivity in construction
141 pits was 62·5%, compared to 5·9% in water cisterns made of cement and 0% in 200L
142 plastic drums (Fig 1B). All abandoned tires sampled did not contain any water.
143 Interestingly, from all the sites that we found positive for *An. stephensi* larvae and
144 pupae, 63.6% of them also had *Culiseta* spp. and *Aedes* spp. larvae in them, whereas
145 only 18·2% and 9·1% sites positive for *An. stephensi* were co-habited by *Culiseta* spp.
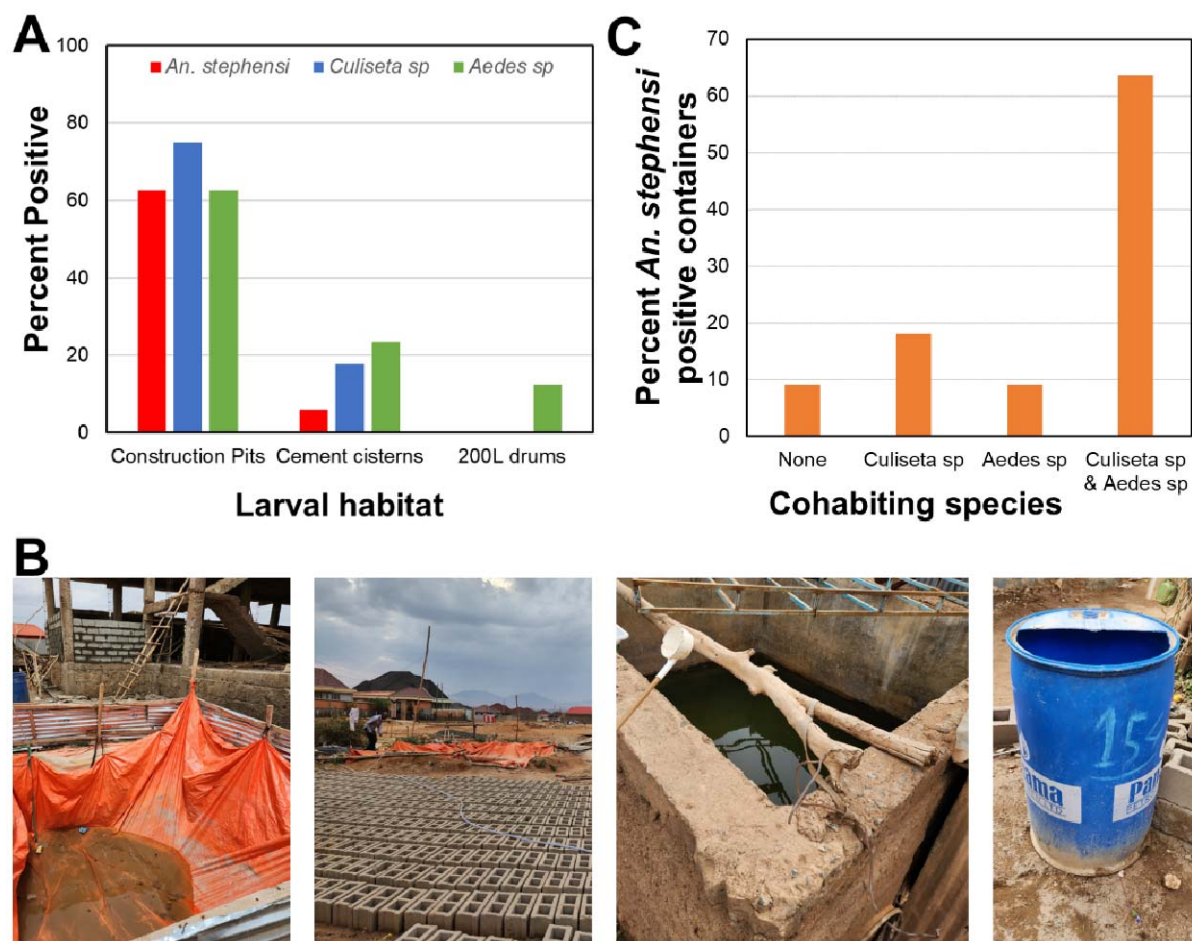146 or *Aedes* spp. only, respectively (Fig 1C).

148 **Figure 1.** (A) Positivity of *Anopheles stephensi* immature stages in the only containers
149 found with water during the dry season survey of 2023 in Jigjiga, Ethiopia, and stratified
150 by species or genus of mosquito found. (B) Examples of sampled habitats, construction

151 pits associated with house construction (left), a construction pit associated with brick
152 manufacturing (center left), a cement cistern (center right) and a 200L plastic drum
153 (right). (C) Species cohabiting with *An. stephensi* in positive containers (*Culiseta*
154 represents *C. longiareolata*, whereas *Aedes* represents *Ae. hirsutus*).

155 A subset of 20 adult emerging from the pupae collected in construction pits and visually
156 identified with standard keys was molecularly confirmed to be *An. stephensi* using an
157 allele specific PCR and the sequencing of *ITS2* and *COI* loci[13]. While *ITS2* haplotypes
158 were all identical for the *An. stephensi* samples, three *COI* haplotypes were detected:
159 Hap 1 (7/14), Hap 2 (6/14), and Hap 3 (1/14) (using Carter et al 2021[25] haplotypes
160 designations), mostly consistent with previous studies. Notably, the presence the *COI*
161 Hap 1 (common to South Asia and detected in northern Ethiopia and Djibouti) supports
162 the notion of Jigjiga's connectivity with regions outside of the continent with long-
163 established *An. stephensi* populations and as a likely entry point for *An. stephensi* into
164 the southern part of the country.

165 After the molecular confirmation of *An. stephensi*, we used the GPS coordinates from
166 the construction pits in Google Earth to identify them remotely, given their unique
167 spectral signature (size, color contrast and presence of water). Interestingly, using a
168 high-resolution satellite image taken in November 2022 (4 months prior to sampling) we
169 not only were able to identify the positive construction pits (Fig 2A-B) but also extended
170 our work to identify a total of 101 pits within a rural to urban swath of Jigjiga centered on
171 the road connecting the city with Somaliland (Fig 2C). The density of pits per hectare
172 within the swath did not follow a rural-urban gradient but concentrated in the center of
173 the swath, an area of Jigjiga currently experiencing rapid construction and development
174 (Fig 2D). Given the high positivity rate of construction pits we quantified (Fig 1), the
175 density map in Fig 4 may be a good proxy for the distribution of *An. stephensi*
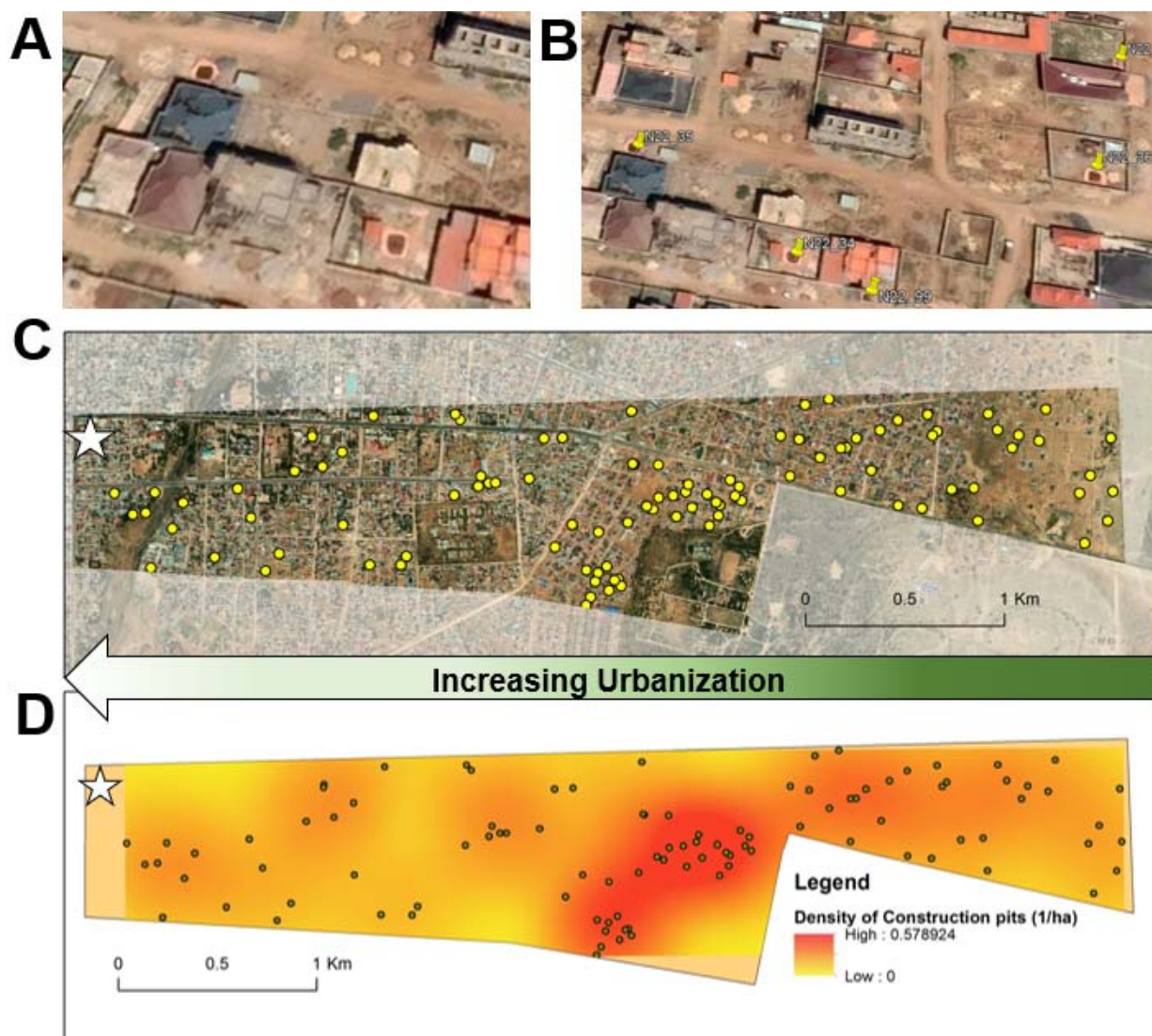176 distribution within the rural-urban swath.

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Figure 2. (A) Construction pits identified as positive for *Anopheles stephensi* larvae during March 2023 in Jigjiga, Ethiopia (visible as two orange squares with darker color in their center representing water). (B) Use of Google Earth Pro to digitize all visible construction pits (in this panel, a total of 5 pits are identified with a pin). (C) Distribution of the 101 construction pits visually identified in November 2022, 4 months prior to our sampling, within a rural-urban swath measuring 4.3 km^2 and centered on the highway connecting Jigjiga with Somaliland (one of the busiest corridors in the region). (D) Kernel density estimate of the density of construction pits per hectare (color surface) and location of all identified pits (dots) using a bandwidth of 500m and a pixel size of 10m. Stars in C and D indicate the location of Jigjiga's downtown.

Political and economic development and *An. stephensi* invasion in Jigjiga. We consider that an unprecedented urban development boom in Jigjiga has been critical in favoring

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192 *An. stephensi* establishment and rapid spread. Jigjiga increased its built-up area from
193 4·2% in 1985 to 5·2% in 2005 to 24·0% in 2015, primarily driven by a change in status
194 from zonal capital to regional capital, which opened political and economic
195 opportunities, leading to high rural-urban immigration[26]. Since 2018, when the most
196 transformative political reform of Ethiopia was enacted by the Ethiopian government,
197 Jigjiga has seen an even larger population and urban footprint increase. The recent
198 declaration by the national government of Ethiopia that 19·0% of commodity imports for
199 the country ought to enter via port Berbera in Somaliland and transported through
200 Jigjiga to the rest of the country[27] led to an increased interest in investment and even
201 higher immigration into the city[27]. Jigjiga's population grew from 125,876 inhabitants in
202 2007 to more than 700,000 in 2020.

203 Since the 2018 political reform in Ethiopia, different groups began to accept Jigjiga's
204 new regional status as a safe regional hub, opening the window of opportunity to
205 increased investment and business development[27]. Diaspora Somalis started to make
206 investments, purchase land, and construct homes leading to a construction boom and
207 increases in the price of land [27]. New hotels, restaurants, as well as businesses are
208 being built in preparation for the increased trade (and truck traffic) with port Barbera [27].
209 As the city continues its unprecedented expansion, it is also increasingly facing critical
210 water shortages (particularly during the dry season); the mean water accessibility of
211 Jigjiga in 2016 was only 19·0% [28]. In response to these water shortages, communities
212 build cement cisterns to store water for domestic uses [28]. Similarly, for building
213 construction or brick manufacturing purposes, people in the town are accustomed to
214 construct temporary construction pits lined with plastic sheet (Fig 1). During the dry
215 season, water for construction pits is generally purchased and delivered in truck
216 cisterns, which source the water from underground wells located outside the city. We
217 can see evidence of the unprecedented construction boom in Jigjiga using historical
218 satellite imagery (Fig 3). From the images one can see the dramatic expansion of
219 construction pits in 2018 as well as the construction further along the periphery of the
220 city. The sector went from 62-84 pits between 2016-2018 to 232 in 2020 and 192 in
221 2021, showcasing the rapid urban expansion of Jigjiga during that time (Fig 3).
222 Construction pits are not only common practice in Jigjiga. In India, it is widely
223 recognized that many *Anopheles stephensi* breeding sites are built into the finished
224 structures of offices, homes and factories in urban areas [29]. Less widely recognized, but
225 also important as *An. stephensi* breeding sites, are the transient structures created
226 during and as part of the construction process [30]. Interestingly, this association between
227 urban development and *An. stephensi* resembles the finding of cutaneous leishmaniasis
228 outbreaks in association with urban growth and, specifically, construction sites in Israel
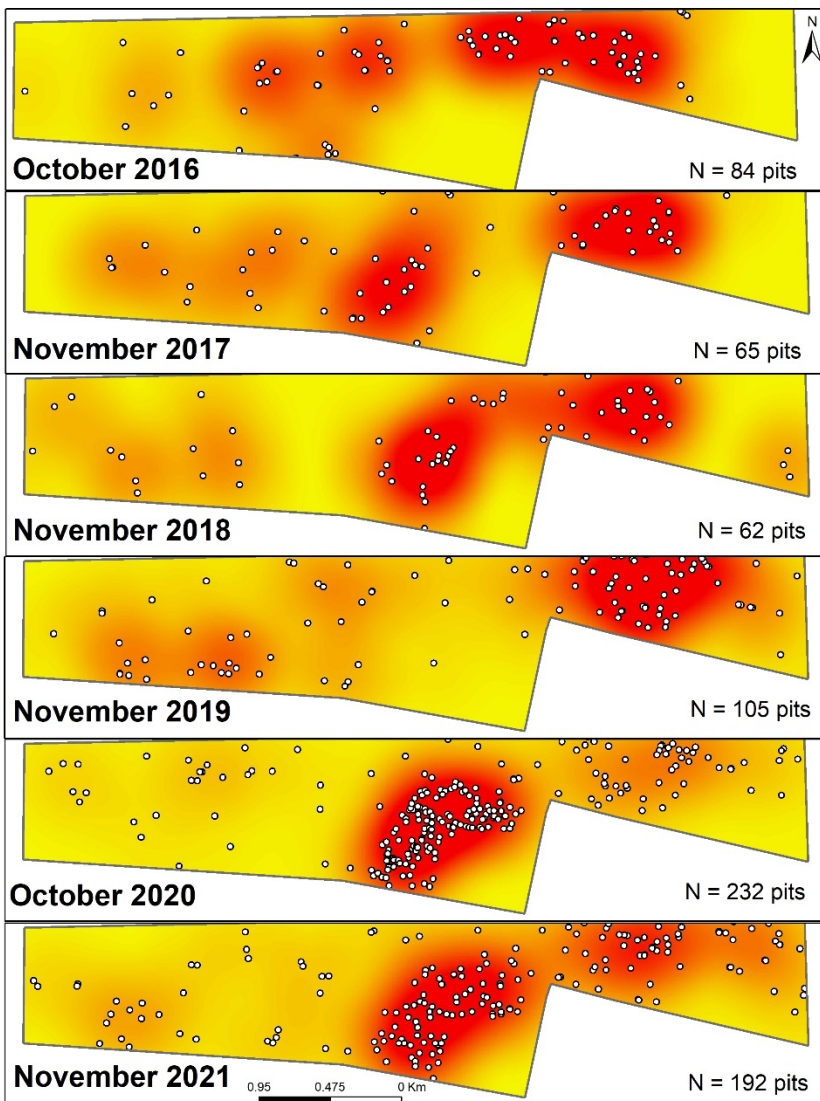229 settlements [31,32].

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Figure 3. Historical sequence of the distribution of construction pits (white dots) in an urban-rural swath of Jigjiga, Somali region, Ethiopia. The dots represent digitized construction pits, observed through high-resolution satellite imagery historically archive in Google Earth. For each year we used October-November, as they were the months that had most complete information. The total number of pits per year on the area is listed on each panel.

Opportunities for *An. stephensi* containment: larval source management of construction pits?. The finding of discrete and easily identifiable *An. stephensi* larval habitats in Jigjiga may provide a unique opportunity for immediate larval source management (LSM) and targeted control during the dry season, particularly with larviciding or biological control. A similar concept of 'dry season LSM' has been proposed for *An. gambiae* in semi-arid Kenya as an approach to maximize the effectiveness of larval control [33]. An extensive list of larvicides prequalified by WHO for vector control exists [34]. While temephos and Bacillus thuringiensis have shown important larviciding effect on

247 *An. stephensi* from Ethiopia [35], they require frequent reapplication, which may be
248 challenging given the number of construction pits that need to be treated. Long-lasting
249 larvicide formulations, that could be potential candidates for control in large water
250 volumes are Spinosad 7·48% DT (Clarke Mosquito Control Products, Inc.) and
251 SumiLarv 2 MR (Sumitomo Chemical Co. Ltd., Japan). Spinosad DT is a tablet for direct
252 application used at the dosage of 0·5 mg/L AI (1 tablet/200 L) for control of container-
253 breeding mosquitoes with a minimal expected duration of optimum efficacy of 4-6 weeks
254 under field conditions [36]. SumiLarv 2 MR is a 2 g plastic disc containing 2% (20 g AI/kg
255 ± 25% w/w) pyriproxyfen used at the dosage of one disk in a water container with a
256 volume of 40 L[37]. Long-lasting methoprene briquettes are commonly used for *Culex*
257 *pipiens* control in catch basins in the US and, if prequalified, would provide an additional
258 long-lasting tool since there is a 6-month extended release formulation (Altosid® 150-
259 Day Briquets, Zoecon)[38].

260 Given the water source and use, long duration of construction pits, and constant
261 availability of water, a biological control option that can be considered is the use of
262 larvivorous fish [39]. Fish that feed on mosquito larvae have been widely used around the
263 world in attempts to control malaria, other mosquito-borne diseases and mosquito
264 nuisance biting [39] and could be used in this case as a "textbook" example [40]. Locally
265 native larvivorous fish exist near Jigjiga[39]. Furthermore, LSM in Jigjiga could include
266 both larviciding and larvivorous fish if larvicides with low toxicity (Spinosad, metoprene
267 or pyriproxyfen) are chosen. More importantly, our finding of high co-habitation between
268 *An. stephensi* with *Culiseta spp.* and *Aedes spp.* mosquitoes provides a unique
269 opportunity for integrated LSM across vectors, which can lead to important co-benefits
270 and a higher justification for the implementation of such programs within Jigjiga and
271 othe cities. Although for malaria typically the emphasis is on the protection of people
272 inside their home (through deployment of ITNs and IRS). In the case of *An. stephensi* in
273 Jigjiga, vectors could be controlled outside the house by conducting LSM during the dry
274 season (the period when mosquito populations are lower and primary larval sites are
275 easier to identify) to reduce the risk of vector establishment and further transmission
276 malaria.

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279 **Concluding remarks**
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281 The spread of *An. stephensi* in Africa may be facilitated in some Ethiopian cities by high
282 urban immigration and an unprecedented construction boom, which is generating novel
283 larval habitats that the vector exploits during the dry season to survive harsh
284 environmental conditions. Our viewpoint emphasizes that the spread and persistence of
285 *An. stephensi* in Jigjiga and other cities in Ethiopia is a planetary health problem that
286 requires a holistic consideration of the environmental, social, and political changes that
287 may be favoring the establishment and onward spread of this major threat to the
288 elimination of malaria from sub-Saharan Africa.

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