Characterisation of the Swarming Behavior of An. coluzzii and An. Gambiae Populations from a Hybrid Zone of Senegal.

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Abstract

**Background** *Anopheles gambiae* s.s. and *An. coluzzii*, two major malaria vectors in Africa, exist in the nature as two incipient, sympatric or allopatric species. In most of their sympatric areas, the reproductive isolation between these two distinct species is thought to be the main barrier to hybridization. However, in Senegal, barriers to the gene flow seems to be leaky in some areas with relatively higher than expected hybridization rates. Here, we characterized the swarming behavior of these two species to investigate its role in the observed high hybridization.

**Methods** The study was carried out in the south and center of Senegal during the 2018 rainy season. Swarms were surveyed at sunset towards the lightest part of the sky, about 0.5–4 m above the ground. Once located, swarm were collected using a net. Indoor resting populations were also collected during the same period from each sentinel village by pyrethrum spray catch earlier the morning. All specimens collected were identified morphologically followed by PCR to estimate the frequency of the two species and female hybrids.

**Results** Results showed that *An. gambiae* swarmed mainly over bare ground whereas *An. coluzzii* swarms over various objects forming a dark-light contrast with the ground. The height of swarms varied between 0.5 to 2.5 meters and the swarming duration was about 10 minutes. Start of swarming was mainly correlated with sunset and no mixed swarms were found in areas of sympatry despite the high level of hybridization rate (2.4% − 4.4%).

**Conclusion** As found elsewhere in West Africa, swarming site segregation is an important pre-mating reproductive barrier between *An. coluzzii* and *An. gambiae* in Senegal. No link was found between swarming behavior and hybridization, but the lack of mixed swarms may be the result of low number of samples obtained in the sympatric area.

**Introduction**

Despite decades of control efforts, malaria remains a major public health problem worldwide. Indeed, the scourge of the disease affects the most the African continent and continues to strike hardest against pregnant women and children (WHO 2019). Furthermore, conventional malaria control interventions, including the front-line vector control tools such as LLINS and IRS, are facing serious
challenges with the widespread of insecticide resistance phenomena in vectors worldwide. Even worse, vectors become more and more resilient to core indoor-based interventions by behavioral changes thus avoiding the contact with insecticide and leading to the increase of the residual transmission when and where “gap in protective coverage” occurs (e.g. outdoor and/or when people are not under bed-net) (Killeen 2013; Moshi et al. 2018). The situation therefore requires the development of innovative control measures in addition to the current interventions to reduce malaria transmission and to drive toward the malaria elimination goal (Bassene et al., 2018; Niang et al., 2018). So far, alternative control methods such as genetically modified mosquitoes and the release of sterile males are likely the two most targeted axes to achieve the eradication objectives (Ito et al., 2002, Dame et al., 2009). Nevertheless, prior to their application, both approaches require a fine understanding of the biology of the targeted species, especially their reproductive behavior and genetic structure (Assogba et al. 2010).

Throughout the sub-Saharan Africa, including Senegal, members of the Anopheles gambiae complex are the main malaria vectors (Coetzee et al. 2013; Niang, et al. 2018; Sy et al. 2018). The complex includes at least 8 sibling morphologically indistinguishable species which emerged through an ecological speciation process. The sympatric ecological diversification process is still ongoing with the recent description of two new incipient species, An. coluzzii Coetzee & Wilkerson sp.n. and An. gambiae Giles, previously considered as molecular forms of the nominal species of the complex (Coetzee et al., 2013). Molecular and genetic studies have shown that in most part of their sympatric distribution range, the hybridization rate between the two incipient species is extremely low, clearly indicating positive assortative mating over their extensive distribution range (Coetzee et al., 2013). However, most recent data in coastal areas at the westernmost of their geographical range reveal a higher than expected hybridization rates (Niang et al. 2014).

The analysis of the pre and post-mating biology and behaviors (respectively assortative mating and larval ecology difference) of the two incipient species across their distribution range suggests that potential pre and post-mating processes may have played a role in the recent divergence between An. gambiae and An. coluzzii (Diabaté et al., 2008; Gimonneau et al., 2012; Thailayil et al., 2018).
Whatever the mechanism involved in the reproductive isolation of these two species, it is likely not as efficient in the westernmost part as in the other regions of their distribution range. Indeed, in several countries of the “Far west Africa”, high levels of hybrids frequencies were reported with 3% in Dielmo (Senegal); 7% in Njabakunda (The Gambia); and 24% in Antula (Guinea-Bissau) (Niang et al. 2014). This suggests the existence of unknown pre and/or post-mating factors breaking down the reproductive isolation between the two incipient species. One of the prevailing hypotheses is that differential swarming behavior favoring heterogamous mating in “Far west Africa” may explain the higher than expected gene flow between the two species (Nwakanma et al., 2013; Niang et al., 2014).

The current study was undertaken to characterize the mating (e. g. swarming) behavior of the two incipient species across their sympatric versus allopatric distribution range, which potentially breaks the reproductive isolation between An. coluzzii and An. gambiae.

Material And Methods

Study area

Three health districts were selected in the center (Fatick) and the south-eastern (Tabacounda and Kedougou) of Senegal. In each of the selected district, two villages were chosen based on their distinctive ecological and entomological features (Fig. 1).

The villages of Koar (13°18'59.6''N, 13°33'50.6''W) and Sare Sidy (13°25'18.9''N; 13°40'48.1''W), in the health district of Tambacounda in the south-eastern Senegal, were selected as a sympatric zone of An. coluzzii and An. gambiae with higher than expected level of hybridization (0.7-6.7 %) (Niang et al., 2014, 2016) compared to elsewhere in Africa (< 1%) (Della Torre et al., 2005). This area is located next to the Gouloumbou River, in a wetland characterized by tree-shrubby savannah and irrigated cultivated landscapes, with the presence of numerous large and permanent water bodies as previously described (Niang et al., 2014, 2016).

The localities of Silly (12°32'33.1''N, 12°16'14.1''W) and Bandafassi (12°32'18.6''N; 12°18'35.4''W) in the south-eastern health district of Kedougou, were chosen as an allopatric area (or area of predominance) for An. gambiae.

Both health districts belong to the Sudano-Guinean climatic domain, where the rainy season lasts
from June to October with a peak in August-September. The area is characterized by an important hydrological system with the Gambia River and its tributaries such as the Gouloumbou River, and where the main activity is banana and rice culture.

In the third health district of Fatick, the localities of Toubanding (13°43’29.7”N, 16°25’24.9”W), and Passy (13°41’56.2”N, 16°23’44”W) were selected as an allopatric area (or area of predominance) for An. coluzzii. The area is located in the coastal area in the center belonging to Sudano-Sahelian climatic domain where the rainy season lasts from June to October with low and irregular rainfall. The study area has the particularity of being crossed by the permanent river of the Nema, creating a specific microenvironment.

**Survey and collection of swarms**

This study was conducted during the raining season in 2018 from August to October in Kedougou and Tambacounda, and from July to October in Fatick. Swarm surveys were done for three successive days per month in each village. Trained operators were hired for the survey and the collection of individuals from each identified swarm. Swarm observations started at sunset and was performed by experienced observers posted towards and checking the lightest part of the sky from 0.5 to 4m above the ground level. Once located, individuals and potential mating pair were sampled from swarms using a net as described by Diabate et al., (2009). The size of all the surveyed swarms was evaluated by estimating the approximative number of swarming males and the number of mating pairs were counted as soon as spotted as they leave the swarm forming a bigger dot easily visible with naked eyes. Upon collection, mosquito specimens and mating pairs were identified morphologically then kept individually in 1.5 ml Eppendorf tubes with silica gel.

During the survey, several parameters related to the swarming behavior were recorded, including their location, the time of the beginning and end of swarming, the size and height above the ground. Once a specific or potential swarm marker is located, it was followed up every evening throughout the villages. Swarms’ locations were georeferenced using a global position system (GPS) then mapped using the QGIS 3.4 software version at a scale of 2 meters.

All the specimens collected (including one mating pair), morphologically identified as An. gambiae s.l.,
were further identified in the laboratory by PCR to distinguish the sibling species of the complex as described by Wilkins et al., (2006) and Santolamazza et al., (2008).

**Characterization of swarm markers**

Since *An. gambiae* mates in flight over physical objects that contrast with the ground or over the ground (Diabate et al., 2009), we therefore recorded in each study village, all the landmarks which could be potential swarm markers the first day of survey. Then all the putative markers were monitored over three consecutive nights per month. All the identified swarms markers were recorded and further characterization for each of the incipient species. To that aim and to better understand the role of physical markers for swarm site selection by the different species, all positive swarm sites were photographed and characterized as described by Diabate et al. (2009). The visual markers used by swarm in different sites were split into five types: bare ground, grass, wood, waste and well (Figure 2A).

**Impact of environmental parameters on swarming start time**

The time of sunset and few environmental parameters such as temperature and relative humidity were recorded every evening right before swarm activities start. A thermohygrometer was used to record the temperature in Celsius degrees and the relative humidity in percentage. The sunset times were obtained from https://www.sunrise-and-sunset.com website.

**Indoor resting collection**

Pyrethrum spray collection was performed indoors in 10 randomly selected human dwellings nearby the swarming points in each of the study villages to estimate the relative frequency of *An. coluzzii*, *An. gambiae* and their hybrids.

The collection was done once a month during the period of survey, the day after the last swarm collection to avoid affecting swarm compositions with the pyrethrum spray. Upon collection all specimens were identified, stored individually in 1.5 ml Eppendorf tube with silica gel and subsequently identified to species level as described by Wilkins et al., (2006) and Santolamazza et al., (2008).

**Data analysis**

Swarming events and their parameters were analyzed comparatively for the two incipient species.
Swarm height, and the swarming duration and markers were compared between An. *coluzzii* and An. *gambiae* using Wilcoxon tests. The size of each swarm was estimated at the peak of swarming event. Swarm duration was calculated as the difference between start and end times of each swarm in minutes. Mean height of swarms above ground, mean size and duration of swarms were also calculated.

A linear regression model was used to test the interaction between swarming start and climatic conditions (sunset time, humidity and temperature). Statistical analysis and correlations tests using Pearson’s correlation test were performed with significance level of 0.05. All the Statistical analysis were done using the R 3.5.2. software version.

**Results**

**Swarm events and collection**

Over the period of the study, swarms appeared usually at the same location every evening, and began to be formed 2-8 minutes after sunset with one or two males acting as precursor and flying tortuously in several directions. As soon as the precursors start swarming, they are joined by other males and sampling was done at the pic of swarm size, 10±2 minutes after the first male was seen. Overall, 197 males were collected from 32 swarms across different areas. Of these, 22 were specific to An. *gambiae*, 6 to An. *coluzzii* and 4 to An. *arabiensis* (Figure 1) with no mixed swarm found over the study area during the study period. However, in this paper further analysis focused only on An. *coluzzii* and An. *gambiae* swarming.

When comparing the swarming behavior of the two incipient species across the study area, only An. *gambiae* swarms (15) were found in Kedougou (10 in Bandafassi (Figure 1A) and 5 in Silly (Figure 1B)), the region chosen as an allopatric area (or area of predominance) for An. *gambiae*. Conversely, only An. *coluzzii* swarms (4) were recorded in Fatick (Passy (Figure 1E)), selected as an allopatric area (or area of predominance) for this species. While in Tambacounda, the sympatric area, swarms of both species were only found in Koar (1 and 2 swarms respectively for An. *gambiae* and An. *coluzzii*) (Figure 1C). In *Sare Sidy*, the second study village in the area, only 5 swarms of An. *gambiae* were found (Figure 1D).
Remarkably, swarming likely occurs either inside (11%) or outside (89%) the houses, and only within the boundaries of the villages where they were observed, since no swarm was recorded far from the villages (Figure 1), despite the effort of investigating the areas beyond their limits.

**Swarm markers and characteristics**

Swarms collected in Kedougou were exclusively of *An. gambiae* species, with overall 74% of the swarms collected over the bare ground and the rest collected over others type of physical markers including grasses (13%) and woods (13%). (Figure 2B). Conversely, only swarms of *An. coluzzii* were collected in Fatick, over physical markers including waste (50%), wood (25%), and grass (25%) as well (Figure 2B). Finally, in Tambacounda, were both species were found, *An. gambiae* swarmed over bare ground while *An. coluzzii* formed swarms both right above bare ground (50%) and wells (50%) (Figure 2B).

In Kedougou, except one swarm of 100 males, the size of *An. gambiae* swarms varied from 5 to 60 males with a mean number of 36 (CI: 22.58-49.41) males (Figure 3A). In Fatick where only *An. coluzzii* was present, the swarms size varied between 5 to 30 males with a mean of 16.25 (CI: -1.39-33.89) (Figure 3A). In their sympatric area in Tambacounda, the mean size of swarms were 35.71 (CI:15.83-55.59) for *An. gambiae* and 25 (CI: -165.59-215.59) for *An. coluzzii* (Figure 3A).

The mean height of swarms was 1.80 m (CI: 1.57-2.04) for *An. gambiae* in Kedougou (Figure 3B) and 1.62 m (CI: 0.86-2.38) for *An. coluzzii* in Fatick (Figure 3B). Significant difference was found between the swarm height of the two incipient species in their respective areas (P=0.00019). Indeed, in Kedougou, where *An. gambiae* was exclusively found, the height of swarms varied between 1 m and 2.5 m, while in Fatick, where *An. coluzzii* was found but not *An. gambiae*, the swarm height ranged between 1 m and 2 m with most of the swarm recorded around 2 m above the ground (Figure 3B).

Finally, in the sympatric area of both species in Tambacounda, the height of swarms varied from 1.5 m to 2 m for *An. coluzzii* against 0.5 m to 2 m for *An. gambiae*. Therefore, the highest swarms being nearly 2.5 m was recorded in Kedougou, while the lowest measured around 0.5 m above the ground recorded in Tambacounda both for *An. gambiae* (Figure 3B). However, the height of swarms of the two species were similar (p= 0.4487) in Tambacounda.
The swarming duration lasted between 7 and 17 min for *An. gambiae* in Kedougou with a median swarming duration of 11 min (9.22-12.77) (Figure 3C). Inversely, it was significantly shorter for *An. coluzzii* (*p*=0.00024), lasting from 5 to 15 min in Fatick with a median swarming duration of 9.75 min (2.95-16.54). However, in the sympatric area in Tambacounda, no significant difference (*p*= 0.6509) was found between the swarming duration of *An. coluzzii* and *An. gambiae*, with respective median values of 8 and 8.42 min (5.66-11.19) (Figure 3C).

**Indoor resting populations species composition**

Collection of indoor resting populations of *An. gambiae* s.l. has been carried out inside randomly selected human dwellings in each site. Overall, 613 specimens of *An. gambiae* s.l. were collected in all sites. Molecular analysis showed that in Kedougou, *An. gambiae* was the predominant species (87.5% in Bandafassi and 92.7% in Silly) compared to its sibling, *An. coluzzii* (12.5% in Bandafassi and 7.3% in Silly) (Table 1). Conversely, in Fatick, *An. coluzzii* (90% in Toubanding and 88.3% in Passy) was predominant over *An. gambiae* (10% in Toubanding and 9.8% in Passy). Interestingly, in Passy few hybrids between the two incipient species were found (1.9%) (Table 1). While in the sympatric zone, in Tambacounda, where the hybridization level was the highest (4.4% in Koar and 2.4% in Sare Sidi), the proportions of the two incipient species were 60.2% in Koar and 83.4% in Sare Sidi, for *An. gambiae* and 35.4% in Koar and 14.2% in Sare Sidi for *An. coluzzii* (Table 1).

**Environmental parameters and swarming start time**

To understand the relationship between some environmental factors and the swarming starting time, temperature, relative humidity and sunset time were recorded during the swarm survey in all the study sites. The temperatures recorded in the different study sites were relatively similar, varying between 28.8°C to 31°C in Kedougou, from 28°C to 32°C in Tambacounda, and from 29.2°C to 29.5°C in Fatick (Figure 4A). Likewise, the relative humidity was higher than 50% in all the study sites, varying from 68% to 84% in Kedougou, 70% to 82% in Tambacounda, and in Fatick 70% to 79% (Figure 4B). Across the study areas, no significant correlation was found neither between the swarming starting time and the temperature (Figure 4A) nor between the swarming starting time and the relative humidity (Figure 4B) (*p* > 0.05), except in Fatick where swarming start time was
significantly correlated with both temperature (figure 4A) and the relative humidity (figure 4B) \((p < 0.05)\).

The sunset times varied according to the study area, occurring earlier in the south-eastern (Tambacounda) and southern (Kedougou) areas than in the Central area (Fatick) (Figure 4C). Overall across the study area, swarming started between 2 to 9 minutes after the sunset, with a significant correlation of the two variables in all the study areas: Kedougou \((R=0.99; P < 0.001)\), Tambacounda \((R= 0.90; P=0.005)\), and in Fatick \((R= 0.99; P=0.001)\) (Figure 4C).

**Swarming behavior and hybridization level**

The level of hybridization recorded in Tambacounda, a sympatric area for the two incipient species, was similar to previous findings with 4.4% and 2.4% of An. gambiae-An. coluzzii hybrids found. Hybridization was also recorded in Fatick (1.9%).

The finding of higher than expected hybridization level in some areas (Tambacounda, 4.4% An. gambiae-An. coluzzii hybrids). However, during the study period, all the swarm collected was 100% monospecific, even in the area of the highest gene flow (Tableau 1).

| Study areas / Sites | Indoor composition | An. gambiae | An. coluzzii |
|---------------------|--------------------|-------------|--------------|
| Tambacounda         | Koar               | 109 (60.22%)| 64 (35.36%)  |
|                     | Sare Sidy          | 206 (83.40%)| 35 (14.17%)  |
| Kedougou            | Bandafassi         | 49 (87.5%)  | 7 (12.5%)    |
|                     | Silly              | 63 (92.65%) | 5 (7.35%)    |
| Fatick              | Toubanding         | 1 (10%)     | 9 (90%)      |
|                     | Passy              | 5 (9.80%)   | 45 (88.3%)   |

**Discussion**

The investigation of the swarming behavior of the natural populations of An. coluzzii and An. gambiae began firstly in Burkina Faso in early 2000s (Diabate et al. 2003, Dabire et al., 2013). Initially only species-specific swarms of either An. coluzzii or An. gambiae were reported with no mixed swarms found by the authors (Diabate et al., 2003a). Indeed, the existence of a strong premating reproductive barrier was based on the temporal and/or spatial disruptive segregation of the two incipient species with no mixed swarms never found up to 2003 (Charlwood et al., 2002; Diabate et al., 2009, 2011). Mixed swarms were first reported from in Burkina Faso in 2004 with (Sawadogo et al., 2013).
In this study, the swarming behavior of *An. coluzzii* and *An. gambiae* was investigated for the first time in Senegal in areas with distinctive ecological and entomological features, selected based on previous *An. gambiae* s.l. species distribution studies (Niang *et al.*, 2014, 2016). Kedougou was chosen as an area of predominance (“allopatric”) for *An. gambiae*, and Fatick likewise but for *An. coluzzii*. Tambacounda, the previously described sympatric area of the two incipient species with a higher than expected level of hybridization (Niang *et al.*, 2014, 2016) compared to the situation across the distribution range of the two species (della Torre *et al.*, 2005), was also selected.

As previously reported swarms were always initiated by one or two males few minutes after the sunset appearing over their preferred swarm maker(s) displaying a tortuous flight pattern. Few minutes later, the precursors were joined by other males with the number of males increasing quickly and reaching their maximum number within 5 minutes after the swarm initiation (Charlwood *et al.*, 2002).

During swarming, female approach swarms to select a mating partner then leave with the selected male in copula (Diabate *et al.*, 2009). In this study only few mating pairs were observed, this may be explained by the low size of the swarms observed, varying between 5 to 60 males for *An. gambiae*, and 5 to 40 males for *An. coluzzii*.

A strong pattern of spatial segregation between *An. coluzzii* and *An. gambiae*, reported from several studies elsewhere has been seen as disruptive species-specific mating events strongly contributing to assortative mating behavior between *An. coluzzii* and *An. gambiae* (Tripet *et al.*, 2001). The results reported here revealed differential swarming behavior between *An. coluzzii* and *An. gambiae* populations according to the study area as shown by Sawadogo *et al.*, (2013) in Burkina Faso, where the swarming behavior of *An. coluzzii* in VK7 differs from those of *An. gambiae* in Soumousso. This doesn’t match with the ongoing gene flow whatever the area under consideration as highlighted by the species composition of the indoor resting population across the study area, even at its lowest rate.

On the other hand, *An. gambiae* population in Kedougou swarms likely earlier than those of *An. coluzzii* in Fatick, probably due to the differences in the environmental parameters such as the sunset
time occurring earlier in Kedougou than in Fatick. However, in the sympatric area of Tambacounda, no significant difference was found between the swarming starting time between the two incipient species. The swarming duration was significantly different between the study populations of *An. gambiae* and *An. coluzzii* according to the area considered. When comparing *An. gambiae* populations in Kedougou against those of *An. coluzzii* in Fatick, the former population swarming lasts longer than the latter. But were almost similar in their sympatric area Tambacounda.

The comparative analysis of both the swarming starting time and its duration, suggest that in the sympatric area of Tambacounda, the similarity of the two species mating characteristics allow the possibility for heterogamous mating, thus explaining the high level of hybridization in this area, despite the fact that no mixed swarm was found yet. While in Kedougou or Factick, where species display a clear differentiated mating time preference and duration as well, which may have led that they were missed one of the sibling species depending on the area under consideration. Furthermore, this may have led for a lesser probability for heterogamous mating as reflected by the hybridization rates in the resting populations of Kedougou and Fatick, where one of the species outnumbered the other from an area to the other.

The analysis of the swarming and the mating behaviors of *An. gambiae* in the field suggests that their males avoid interspecific contact when searching for mating partners through mainly disruptive swarming height above the ground (Charlwood *et al.*, 2002). Likewise, the present study revealed significant difference between the swarming height between *An. gambiae* and *An. coluzzii*, notwithstanding when considering them in the area where only the swarm of a single species was found (Kedougou: *An. gambiae* or Fatick: *An. coluzzii*) or where both were found swarming (Tambacounda). Indeed, *An. gambiae* appeared significantly in higher heights in Kedougou than *An. coluzzii* in Fatick. This finding contrast with those from Sawadogo *et al.*, (2013) which showed that the swarming height of *An. gambiae* was significantly lower than that of *An. coluzzii*. This suggests that the swarming behavior of species could change according to the localities with the adaptation to the environmental conditions as demonstrated by Marchand (2004) who observed a difference of the
swarming behavior in Sao Tomé and in Tanzania.

Swarms have been reported to form at varying heights as well possibly determined by species-appropriate swarm markers or related to orientation with swarm markers (Marchand 1984; Charlwood et al., 1980, 2002, 2003). *An. gambiae*, the major malaria vector in Africa swarms over specific landmarks known as swarm markers (Charlwood et al., 2002; Diabate et al., 2003a, 2009; Yuval 2006). The disappearance of swarms in unusual areas where the artificial markers that they have been following were removed indicates that visuals cues were the only one involved in swarm maintenance (Charlwood et al., 2002). More recently, Poda et al., (2019) have demonstrated both in laboratory and semi-field conditions that males of the two incipient species both use visual markers but differently with *An. coluzzii* swarming right above markers while its counterpart, *An. gambiae* use the maker but swarm above bare-ground at a constant distance.

Our results support the role of swarm markers as a determinant of swarm segregation between *An. coluzzii* and *An. gambiae*. The swarm marker of *An. coluzzii* and *An. gambiae* in their respective areas were different depending to the area under consideration. In Kedougou, *An. gambiae* was found swarming mainly over the bare ground and accidentally over dry grasses or wood, potentially keeping a constant distance from a maker, as suggested by Poda et al. (2019), which was unfortunately not investigated during the present study. The fact that the same swarming point were kept by *An. gambiae* despite the change that occurred over the time with the first record and georeferencing of the point made as bare ground which then become covered by grass support the hypothesis that a constant maker yet to be found may exist and that would be used as a landmark by the swarming males of *An. gambiae*.

Conversely, in Fatick males of *An. coluzzii* were found swarming either over grasses, waste and wood piles, but none over the bare ground. These results support previous studies where an allopatric S form (now known as *An. gambiae*) population in Tanzania swarmed exclusively on bare ground (Marchand 1984) whereas an allopatric M form (e.g. *An. coluzzii*) population in Sao Tomé used patterns of contrast as markers (Charlwood et al., 2002). Similar results were reported by Diabate et al., (2009). Nevertheless, in the sympatric area, despite the fact that *An. gambiae* was found
exclusively swarming over the bare ground, *An. coluzzii* did not display the same restrictive preference in terms of swarm marker since it was found both above bare ground and well.

The occurrence of crepuscular swarms of *An. coluzzii* and *An. gambiae* appears to be controlled predominantly by the sunset time over the season, with swarms formed with the reduction of the light toward the sunset. Sawadogo *et al.*, (2013) showed results suggesting that a biological clock regulate the timing of swarming activity of the species which allow them to adjust themselves to cyclic changes in day length and the timing of sunset through the year. The correlation of the environmental parameters and the swarm starting time showed a significant correlation between the swarm apparition and sunset time. Whatever the area of interest, swarm appears 2 to 9 minutes after the sunset. However, the preliminary results support the findings reported by Sawadogo *et al.*, (2013), as stated above. Furthermore, the authors did find also a significant correlation between the relative humidity and swarm starting time, and sometime even between the temperature and swarm starting time in the same study likewise in Fatick, where the swarming starting time was significantly correlated with the temperature and the relative humidity. While, in Kedougou and Tambacounda no significant correlation was found between the combination temperature-humidity and swarm starting time suggesting antagonist effects of the two climatic factors. However, further conclusion cannot be made in comparison to Sawadogo *et al.*, (2013), given the shorter study period of the current study compared to the year-round of the previous one.

The predominance or the exclusive presence of one species in a given area may depend on their ability to adapt and survive in that area (Sawadogo *et al.*, 2013). *An. coluzzii* and *An. gambiae* differ in their ecological preference both at the larval or adult stages (Gimmoneau *et al.*, 2012, thus explaining their spatial and temporal distribution (Coluzzii *et al.*, 1979; coluzzii 1999; Touré *et al.*, 1998). *An. gambiae* larvae are found in rain-dependent surface water bodies/puddles while those of *An. coluzzii* are more adapted to more permanent anthropogenic breeding sites such as irrigated rice fields (Diabate *et al.*, 2003, 2005; Torre *et al.*, 2005). Furthermore, *An. coluzzii* larvae display a greater tolerance to aridity and even organic pollution (Kamdem *et al.*, 2012). Which may explain its predominance in arid areas and at the vicinity of human settings such as urban area, thus
corroborating our finding of *An. gambiae* predominating in rural and most humid areas (Kedougou and Tambacounda), while *An. coluzzii* was the most prevalent in the arid area Fatick.

Spatial swarm segregation which is one of the better characterized mechanisms of pre-mating reproductive isolation was reported in Mali and Burkina Faso (Diabate *et al.*, 2009; Sawadogo 2013), where no or very low frequencies of hybrids were found; suggesting that the spatial swarm segregation could play the role of the first barrier to hybridization. In contrast, despite no mixed swarms was found yet, a relatively high hybridization (4.4%) in Tambacounda suggest that the heterogamous mating. This observed hybridization without no record of mixed swarm could be explained on one hand by the fact that despite the significant difference of the swarming behavior between *An. coluzzii* and *An. gambiae* in their respective areas and no difference was found in their sympatric area these may have been missed probably occurring where and when they were not expected. And, on the other hand, possibility through indoors mating in the absence of any form of conspecific recognition as demonstrate by Dao *et al.*, (2008). Therefore, to link the swarming behavior and hybridization rate, Sawadogo *et al.*, (2013) which found no hybrids from indoor sample or mating couples despite the high frequencies of mixed swarms suggest that the occurrence or absence of mixed swarms is not necessarily the main pathway for the two incipient species hybridization. Therefore, further investigations need to be undertaken considering all the above hypothesis to better understand the underlying factor of the “Breakdown in the Process of Incipient Speciation in *Anopheles gambiae*” at its furthermost “Far-west” distribution range (Niang *et al.*, 2014, Nwakanma *et al.*, 2013).

**Conclusion**

This study provides the first evidence on the swarming behavior of the natural population of *An. coluzzii* and *An. gambiae* in Senegal. Despite, preliminary, the results reported here reveal a possible pre-mating isolation between the two incipient species. However, the analysis of the species composition of the resting populations across the study area suggests that heterogamous mating still occurring during time at place yet to be known. Despite preliminary, the data reported here are crucial for the design of future studies to better characterize the mating behavior of the two incipient
species to better support current and future vector control method, including the release of sterile males.

List Of Abbreviations
IRS = Indoor Residual Spraying; LLINs = Long Lasting Insecticide Nets; WHO = World Health Organisation; PCR = Polymerase Chain Reaction.

Declarations
Ethics approval and consent to participate: This study was approved by the Ethics committee of University Cheikh Anta DIOP of Dakar, Senegal.

Consent for publication
Not applicable

Availability of data and material
The datasets generated and/or analysed during the current study are available from the corresponding author upon request

Competing interests
The authors declare that they have no competing interests.

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Authors' contributions
OKG, MBF and AAA undertook the field and laboratory work. OKG, AKD, MBF, AAA and EAN analysed the results. OKG, AKD and EAN drafted the manuscript. FT, CW, AD and EAN designed the study. FT, ID, CW, AD, KL, OG, FO and EAN reviewed the manuscript. All authors read and approved the final
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Figures

Figure 1

Study areas. The letters A-F refer to the putative swarms location of *An. gambiae* s.l. species surveyed over the time in each of the study region.
Figure 2: Swarming markers used by An. coluzzii and An. gambiae across the study area

Swarming markers used by An. coluzzii and An. gambiae across the study area. A) Pictures of the different swarming makers found in the study areas. B) Proportion of each swarming maker by species and study area.
Swarming size (A), height (B) and duration (C) of \textit{An. coluzzii} and \textit{An. gambiae} across the study area.

Figure 3

Swarming size (A), height (B) and duration of \textit{An. coluzzii} and \textit{An. gambiae} across the study area.

Figure 4

Relationship between the temperature (A), relative humidity (B), sunset time (C) and the swarming start time.

Figure 4

Relationship between the temperature (A), relative humidity (B), sunset time and the swarming starting time.
Supplementary Files

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