

# Potential risk for the introduction of exotic mosquito species to Germany via transnational vessels

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## Abstract

In a globalized world, different invasive, highly vector-competent mosquitoes like the Asian tiger mosquito or the Yellow fever mosquito are transported by human assistance all over the world. At the global scale, this spread can be attributed to trade and transport via oceanic vessels or international air traffic. To conform to the International Health Regulations (WHO), the city of Hamburg (Germany) was designated a Point of Entry. In order to assess the potential risk for the introduction of exotic mosquito species via transnational shipping, five mosquito traps (Biogents-Sentinel 2<sup>®</sup> trap with BG-lure) were installed on five different sites of a container vessel, starting in Hamburg shipping via West Africa to several South American Atlantic harbours with one to two days of stay in each port, returning to Hamburg via West Africa.

The traps were operated continuously from departure until the return of the vessel to Hamburg after 67 days. Samples were taken once at the end of the journey. Supported by a DNA barcoding approach (mitochondrial COI gene), seven of 128 collected dead mosquito specimens were identified as *Aedes aegypti* and four as *Anopheles gambiae* s.l. Additionally, 19 specimens were classified as *Culex pipiens* cf. *quinquefasciatus* using a DNA typing assay. These results indicate the potential risk for the introduction of exotic mosquito species to Germany via transnational vessels and highlight the need of monitoring of seaports as potential introduction sites of invasive mosquito species. However, the question of the mosquito survival probability on the ships needs further investigation.

## Methods

We chose a shipping company where the contact from the Hamburg Port Health Center had intensified due to the Ebola crisis 2014 (a vessel takes only 10 days from West Africa to Hamburg). The identified company serves South America and Hamburg on one single route. On a large Roll-on/roll-off cargo ship which operates between Northern Europe, Hamburg, West Africa and Brazil, we were allowed to set up five adult mosquito traps (Biogents-Sentinel 2<sup>®</sup> trap with BG-lure, Fig. 1). These traps do not need CO<sub>2</sub> gas to be run, which could be difficult to be on use on a traveling vessel. Locations were chosen that were at the same time in the vicinity of people and of electric devices. Two traps were installed at the bottom opening of the stern ramp and three in the vicinity of the quarters in the superstructures: pantry, hospital room and navigation bridge (Fig. 2). The traps were operated continuously from departure until the return of the vessel to Hamburg. The only possibility to empty the traps was at the end of the trip.

Mosquito specimens were morphologically identified [Becker et al. 2010]. The specimens were strongly damaged and missed relevant characters for the species identification. Therefore, the specimens classified as *Cx. pipiens* s. l./*torrentium* were identified to the species level (*Cx. pipiens pipiens* form *pipiens*, *Cx. pipiens pipiens* form *molestus* and *Cx. torrentium*) using a molecular DNA typing assay [Rudolf et al. 2013]. For all other specimens, a molecular identification was conducted by the analysis of the mitochondrial cytochrome c oxidase subunit I gene [Kambhampati & Smith 1995]. PCR products were sequenced by conventional Sanger technology (LGC, Berlin, Germany).

## Objective

The International Health Regulations (World Health Organization 2005) are subject to implementation all over the world. Countries have designated locations and borders with high probabilities for the import of infectious agents or vectors. These so called 'Points of Entry' (POE) can be harbors, airports or ground crossings and in Germany, Hamburg is one of the designated POEs.

In the context of the increasing spread of the invasive exotic mosquito *Aedes albopictus* and its import even into southern Germany by train and road (Becker et al. 2013), together with the Zika epidemic in the Americas 2015 /2016 and the rising numbers of Arbovirus-infections all over the world, a harbor town with thousands of ships visiting each year has a special concern on surveillance.

Hamburg is the 17th largest container port worldwide with a total seaborne cargo throughput of 138.2 million tons and about 9,000 arrivals of ocean-going vessels in 2016. In order to assess the potential risk for the introduction of exotic mosquito species via transnational shipping into Hamburg, a vessel travelling to countries endemic for Zikavirus as well as *Aedes mosquitoes* and back to Hamburg was supplied with mosquito traps on its journey.

Table 1: Route of the vessel Grande Nigeria, August until October 2016

Port	Arrival	Departure
Hamburg	24.10.2016 21:24	25.10.2016 23:15
Banjul	13.10.2016 23:30	15.10.2016 09:00
Dakar	12.10.2016 10:53	13.10.2016 11:53
Vitoria	04.10.2016 21:50	05.10.2016 20:15
Rio de Janeiro	03.10.2016 11:15	03.10.2016 23:54
Santos	30.09.2016 13:20	01.10.2016 07:55
Paranaagua	29.09.2016 13:52	30.09.2016 00:48
Montevideo	22.09.2016 11:11	23.09.2016 14:15
Zarate	19.09.2016 13:48	21.09.2016 08:55
Santos	15.09.2016 18:55	16.09.2016 06:09
Rio de Janeiro	14.09.2016 13:01	15.09.2016 00:02
Vitoria	12.09.2016 17:31	13.09.2016 16:38
Dakar	03.09.2016 10:14	05.09.2016 03:51
Vigo	29.08.2016 06:36	29.08.2016 14:10
Antwerpen	23.08.2016 18:40	25.08.2016 21:22
Tilbury	21.08.2016 19:07	23.08.2016 03:13
Hamburg	18.08.2016 19:36	19.08.2016 22:13

[https://www.vesseltracker.com/de/Ships/Grande-Nigeria-9246580.html?show=berth\\_history#block](https://www.vesseltracker.com/de/Ships/Grande-Nigeria-9246580.html?show=berth_history#block)

Fig. 1: Biogents Sentinel 2<sup>®</sup>-Mosquito trap



Table 2: Trap location, species and number of mosquitos caught on the vessel

	<i>Aedes</i> sp.	<i>Aedes aegypti</i>	<i>Anopheles gambiae</i>	<i>Culex</i> spp.	<i>Culiseta annulata</i>	Total no. trapped
Trap 1 (left of ramp)	1	7	3	104	1	116
Trap 2 (right of ramp)	0	0	1	8	0	9
Trap 3 (pantry)	0	0	0	1	0	1
Trap 4 (hospital room)	0	0	0	0	0	0
Trap 5 (navigation bridge)	0	0	0	2	0	2
<b>Total</b>	<b>1</b>	<b>7</b>	<b>4</b>	<b>115</b>	<b>1</b>	<b>128</b>

## Results

The ship left Hamburg on August 18, 2016 and traveled in 67 days from Hamburg via West Africa to several South American Atlantic harbors with one to two days of stay in each port. The route is indicated in Table 1. The temperature ranges at most of the harbor stops in South America were between 19 and 27 degrees Celsius and were followed via [www.wetter.de/brasilien](http://www.wetter.de/brasilien). The 5 adult traps that had been installed on the ship were in good condition at the time of removal.

Most of the mosquitoes were trapped at the open ramp. The specimens were strongly damaged. The distribution of the trapped mosquitos is shown in Table 2. All in all 128 specimens of dead mosquitos could be sorted from the nets of the traps, 104 of them being *Culex* spp.. Of them, 19 specimens were classified as *Culex pipiens* cf. *quinquefasciatus*. Eight *Aedes* mosquitos were caught in trap No. 1, located at the left bottom side of the stern ramp (7 *Aedes aegypti* and 1 *Aedes* sp.). Additionally 3 findings of *Anopheles gambiae* s.l. were recorded in trap No. 1. Another *Anopheles gambiae* s.l. was caught in trap No. 2, which was situated in a commando room at the right bottom side of the stern ramp, together with 8 *Culex* individuals. Only in one of the three traps in the superstructures, mosquitos were caught (2 *Culex* sp. in trap No. 5 on the bridge).

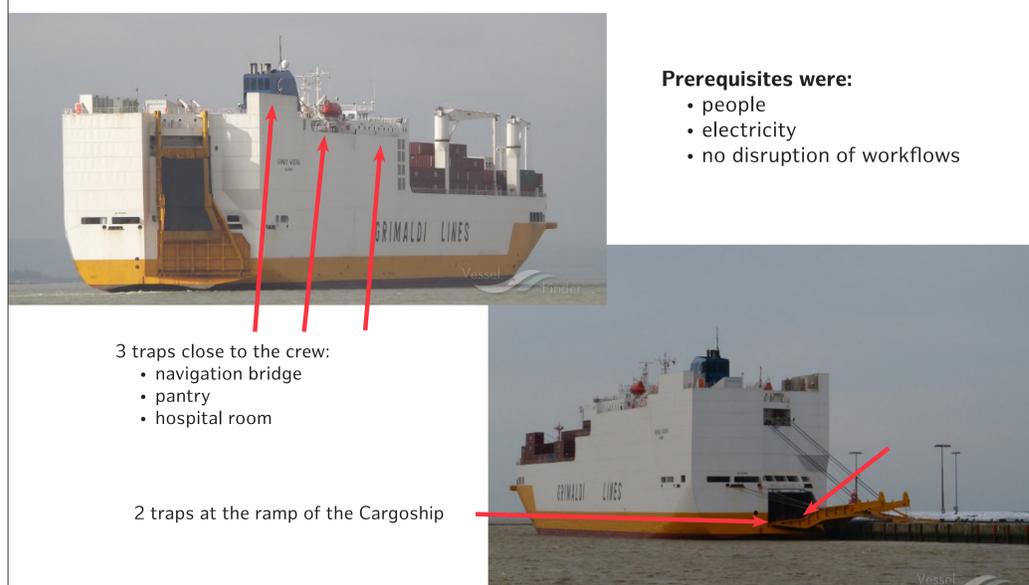
## Conclusion

This first trial resulted in evidence that mosquito vectors enter large vessels and give the prerequisites for them to be transported to Hamburg. They highlight the need of monitoring of seaports as potential introduction sites of invasive mosquito species. However, the question of the mosquito survival probability on the ships needs further investigation. Also there was no way of analyzing a possible viral load of the mosquitos. Therefore a coming further investigation will trap more intensively and constantly on the journey of a vessel heading for Hamburg with the possibilities of a daily morphological analysis and freezing of the trapped species for later virological investigations. Choosing a traveling time more towards the mosquito season will result in more mosquito individuals.

## References

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Fig. 2: Locations of mosquito traps on the Roll-on/roll-off cargo ship



### Prerequisites were:

- people
- electricity
- no disruption of workflows

3 traps close to the crew:

- navigation bridge
- pantry
- hospital room

2 traps at the ramp of the Cargoship