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Vector competence of *Culex antennatus* and *Anopheles coustani* mosquitoes for Rift Valley fever virus in Madagascar.

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1 **Vector competence of *Culex antennatus* and *Anopheles coustani* mosquitoes to Rift**
2 **Valley Fever Virus in Madagascar**

3

4 **Running head:** Vector competence of VRVF in Madagascar

5

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32 **Abstract**

33 *Culex antennatus* (Diptera: Culicidae), *Anopheles coustani* (Diptera: Culicidae) and
34 *Anopheles squamosus/cydippis* (Diptera: Culicidae) were found infected with Rift Valley
35 Fever virus (RVFV) during an epidemic that occurred in 2008-2009 in Madagascar. To
36 understand the role played by *Cx. antennatus* and *An. coustani* in maintenance and
37 transmission, we assessed RVFV vector competence of these two species. Mosquito body
38 parts and saliva of mosquitoes that fed on RVFV-infected blood were tested for RVFV using
39 real-time (RT-PCR) assays. Overall, we detected viral RNA virus in body parts and saliva at 5
40 days post infection (dpi) for both species. At 5 dpi, infection rates were 12.5% (3/24) and
41 15.8% (6/38), disseminated infection rates were 100% (3/3) and 100% (6/6), transmission rate
42 were 33.3% (1/3) and 83.3% (5/6), and transmission efficiencies were 4.2% (1/24) and 13.2%
43 (5/38) respectively for *Cx. antennatus* and *An. coustani*. Although RVFV detected in saliva
44 did not propagate onto Vero cells, these results support a potential role of these two-mosquito
45 species in the transmission of RVFV.

46

47 **Key-words:** Risk assessment, Arboviruses, Emergence, Madagascar

48

49 **Introduction**

50 Rift Valley Fever Virus (RVFV) belongs to the *Phlebovirus* genus and *Bunyaviridae* family.
51 It can cause severe and fatal illness in domestic animals (Woods et al. 2002). Humans can
52 develop an encephalitic, ocular or hemorrhagic syndrome (Kahlon et al. 2010). RVFV can be
53 transmitted by close contact with infectious tissues or through mosquito infectious bites
54 (Smithburn et al. 1948). Its geographical distribution has extended and it could emerge in an
55 area previously not known to have RVFV transmission (Balenghien et al. 2013).
56 In Madagascar, RVFV was detected for the first time in 1979, from a pool of multi and
57 monospecific mosquitoes caught in a rainy forest of the central eastern part of the island
58 (Fontenille et al. 1988). The monospecific pool was composed of *Mansonia uniformis*
59 (Diptera: Culicidae). Surprisingly, the virus was not detected neither in human nor in animals
60 (Clerc & P 1981). Since this first detection, RVFV reemerged in 1990-91 and 2008-09,
61 resulting in outbreaks that affected both animals and humans (Morvan et al. 1992; Fontenille
62 et al. 1989; Andriamandimby et al. 2010). Furthermore, during the last epidemic, RVFV was
63 detected in three mosquito species: *Culex antennatus*, *Anopheles coustani* and *Anopheles*
64 *squamosus/cydippis* (Ratovonjato et al. 2011). These mosquito species were also part of the
65 pool of mosquitoes examined in 1979 (Clerc & P 1981).
66 Twenty-four Malagasy mosquito species are potentially associated with RVFV transmission
67 in Madagascar (Tantely et al. 2015). These species belong to the genera of *Aedes*, *Anopheles*,
68 *Culex*, *Eretmapodites*, and *Mansonia*. All these mosquitoes feed preferentially on domestic
69 animals (cattle, sheep, and goat) but can also have opportunistic anthropophilic behavior
70 (Tantely et al. 2015).
71 Despite a recurrent circulation of RVFV (Andriamandimby et al. 2010; Ratovonjato et al.
72 2011; Gray et al. 2015) and the abundance of potential vectors (Tantely et al. 2015), no study
73 on vector competence has been carried out to date in Madagascar. To better understand the

74 mechanism of maintenance and transmission of RVFV in Madagascar, we performed a study
75 aiming to evaluate the vector competence of *Cx. antennatus* and *An. coustani*, two mosquito
76 species implicated in the last RVFV outbreak in Madagascar.

77

78 **Materials and Methods**

79 **Mosquito collection and rearing**

80 Engorged and gravid females of *Cx. antennatus* and *An. coustani* were captured in three
81 different sites of Madagascar where RVFV has been detected in cattle and human during the
82 2008-09 outbreaks (Figure 1).

83 Mosquito females were either captured in stables early in the morning or in zebu pens using
84 oral aspirators. Trapping in zebu pens was conducted from 06:00 pm to 06:00 am for two
85 consecutive days. After capture, mosquitoes were kept alive in cages with free access to a 6%
86 sugar solution. Upon arrival to the laboratory, mosquitoes were identified morphologically
87 and then maintained separately in breeding cages according to species. After laying, eggs
88 were immersed in 1.5 cm depth of dechlorinated water. Immature stages were then separated
89 and reared until the adult stage. Emerged adults were placed in cages up to 300 individuals
90 per cage. First generations of females (F1) aged from 3 to 5 days were used for vector
91 competence experiments.

92 **Virus for experimental infections**

93 The virus strain used in this study was isolated from a RVFV-infected pool of mosquitoes
94 collected during the 2008-09 outbreak in Fianarantsoa (Ratovonjato et al. 2011). Viral stocks
95 were prepared after three passages of the isolate on Vero E6 cells. Stocks were produced in
96 12-well tissue culture plates maintained at 37°C. After 72h, the supernatant was collected and
97 kept at -80°C. Viral titer was estimated by plaque reduction assay on Vero cells. For all
98 assay, we used a viral titer of 2.25×10^8 plaque-forming units (pfu)/mL.

99 Artificial infection of mosquitoes

100 Artificial infection experiments were performed with the Hemotek membrane feeding system
101 (Hemotek Ltd, UK). An infectious blood meal was prepared according to the protocol of
102 Moutailler et al. (Moutailler et al. 2007). Briefly, the infectious blood meal consisted of 2/3 of
103 erythrocytes (2 mL) and 1/3 (1 mL) of a viral suspension with ATP used as a phagostimulant
104 at a final concentration of 5×10^{-3} M. Mosquitoes were presented with the opportunity to
105 blood feed for a maximum of 30 min. Fully engorged female mosquitoes were sorted on ice
106 and maintained at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$, humidity: $80\% \pm 10\%$, photoperiod: 12 h/12 h and then
107 provided with 6 % sucrose solution for 14 days. Experimental infections were conducted in
108 the Biosafety Laboratory Level-3 at the virology unit and were repeated three times.

109 Virus detection

110 Viral detection in mosquitoes was conducted at 2, 5, 8 days post-infection (dpi) for *An.*
111 *coustani* and at 2, 5, 8 and 14 dpi for *Cx. antennatus*.
112 Viral detection was carried out for the midgut, head and thorax, saliva, legs and wings. Except
113 saliva, all other samples were ground with a TissueLyzer in 200 μL of medium MEM
114 containing 40% SVF. Then, 140 μL of saliva and 140 μL of homogenate obtained from other
115 mosquito tissues/organs were used to extract viral RNA using an extraction Kit Nucleospin®
116 Dx Virus (Macherey-Nagel, Mauritius). RNA was eluted in a final volume of 50 μL in H_2O
117 RNase Free. Presence of RVFV was tested by real-time quantitative RT-PCR (Bird et al.
118 2007). Primers amplified a fragment of 90 base pair (bp) of the L segment encoding for the
119 viral polymerase.

120 Analyses for vector competence

121 Four parameters were studied and analyzed at each dpi: (a) the infection rate (IR) indicating
122 the proportion of mosquitoes with infected midgut among tested mosquitoes; (b) the
123 disseminated infection rate (DIR), corresponding to the proportion of individuals with

124 infected head, thorax, legs or wings among mosquitoes with midgut infected; (c) the
125 transmission rate (TR) showing the proportion of individuals with infectious saliva among the
126 mosquito with disseminated infection (i.e. individuals with infected head, thorax, legs or
127 wings); and finally (d) the transmission efficiency (TE), defined as the proportion of
128 individuals with infectious saliva among the total number of mosquitoes tested (Chouin-
129 Carneiro et al. 2016).

130 To test if RVFV detected in mosquito saliva can propagate in cells as a proxy of the presence
131 of infectious virions in the saliva, 100 μ L of saliva were inoculated on Vero E6 cells. RVFV
132 from viral stocks were also inoculated as positive control. If a cytopathic effect was observed
133 on cells after inoculation, then the saliva was considered as infectious containing replicating
134 viral particles. The extrinsic incubation period (EIP) corresponding to the duration between
135 the infectious blood meal and the first detection of the virus in mosquito saliva was estimated
136 for each mosquito species.

137

138 **Results**

139 Among the 216 *Cx. antennatus* individuals fully engorged, 83 (38. 4%) were tested for the
140 presence of virus in the midgut. The remaining 133 mosquitoes died before the period of
141 testing. Values of IR, DIR, TR and TE are presented in Table 1. RVFV was detected in the
142 head, thorax, legs, wings and saliva at 5 dpi (Table 1). We began to detect the IR at 2 dpi with
143 40% (4/10) of individuals tested and DIR, TR and TE at 5 dpi. At 5 dpi, values of IR, DIR,
144 TR and TE were 12.5% (3/24), 100% (3/3), 33.3% (1/3) and 4.2% (1/24) respectively. DIR
145 reached 100% from 5 dpi i.e all individuals with infected midgut were also infected in head,
146 thorax, legs and wings. Thus, for *Cx. antennatus*, EIP was estimated to be 5 days and 4.16%
147 of individual tested had infected saliva.

148 For *An. coustani*, among 232 individuals fully engorged, 64 (27.6%) were tested for the
149 presence of virus in midgut. The remaining 168 mosquitoes died before the period of testing.
150 Values of IR, DIR, TR and TE are presented in Table 2. RVFV was detected in head, thorax,
151 legs, wings and saliva at 5 dpi (Table 2). IR values have been beginning detected at 2 dpi with
152 15% (3/20) of individuals tested and DIR, TR and TE at 5 dpi. At 5 dpi, values of IR, DIR,
153 TR and TE were 15.8% (6/38), 100% (6/6), 83.3 (5/6) and 13.2% (5/38) respectively. DIR
154 reached 100% from 5 dpi i.e all individuals with infected midgut had virus detected in head,
155 thorax, legs and wings. Thus for *An. coustani*, EIP was estimated to be 5 days and 13.2% of
156 individual tested had infected saliva.

157 From both *Cx. antennatus* and *An. coustani*, RVFV detected from saliva using real-time
158 quantitative RT-PCR did not propagate onto Vero cell.

159 **Discussion**

160 *Anopheles coustani* and *Culex antennatus* collected in Madagascar were tested for their vector
161 competence to RVFV. Results showed that 4.2% and 13.2% of *Cx. antennatus* and *An.*
162 *coustani* respectively, were susceptible to RVFV infection with viral RNA detected in saliva.
163 These results were consistent with descriptions of *An. coustani* and *Cx. antennatus* infected
164 with RVFV in natural mosquito populations (Hanafi 2011, Ratovonjatovo 2011, Seufi &
165 Galal 2010). Most importantly, we detected RVFV in saliva of both species which is a crucial
166 information in vector competence assays as it shows that mosquitoes could transmit the virus
167 to vertebrates during feeding.

168 The virus detected in saliva did not propagate onto Vero cells. These results could be
169 explained by the sensitivity of our cell system that required a higher titer of virus in the saliva.
170 Experimental infections of vertebrate (rodent) with infected mosquitoes is needed to
171 demonstrate that viral particles detected in saliva are indeed infectious.

172 In our experiments, the EIP were 5 days for both *An. coustani* and *Cx. antennatus* which is
173 consistent with previous work from Turell et al. In this study, authors observed an EIP for *Cx.*
174 *antennatus* ranging from 3 to 10 dpi according titers of RVFV used (Turell et al. 2008).
175 Nevertheless, while they estimated the transmission efficiency at 84% (Turell et al. 2008), in
176 our case, using a similar viral titer of 10^8 pfu/mL, we obtained a transmission efficiency of
177 only 4.2%. This difference could be explained by the difference of viral strain used, genetic
178 background of mosquito population, and mode of infection (oral vs. intrathoracic inoculation)
179 (Turell et al. 2008).

180 Although *An. coustani* species have been detected positive for RVFV in nature, we showed
181 for the first-time evidence of replication of RVFV in salivary glands of *Anopheles* species.
182 Indeed, previous studies on *An. pharoensis* from Egypt and *An. stephensi* from laboratory
183 strain (US Army Bioengineering Research and Development Laboratory) never observed
184 release of RVFV in the saliva (Turell et al. 1996; Turell & Romoser 1994).

185 These findings highlight the association of *An. coustani* and *Cx. antennatus* with RVFV in
186 nature. This association between *An. coustani* and *Cx. antennatus* and some vertebrate hosts
187 of RVFV has been underlined in different regions of Madagascar. Indeed, these species were
188 found mostly zoophilic especially *Cx. antennatus* and attracted by goat, sheep and zebu in two
189 regions geographically distant in Madagascar (Nepomichene et al. 2015). It has also been
190 shown that *An. coustani* rest in stables during the day and analyses of ingested blood meal
191 showed that this species has a trophic preference for zebu. Nonetheless, this species was also
192 found to feed on human (Nepomichene, Tata, et al. 2015).

193 To conclude, *An. coustani* and *Cx. antennatus* were previously found associated with RVFV
194 in nature in Madagascar and in other countries. The vector competence described herein by
195 the four indices showed that the two species are susceptible to RVFV and can potentially
196 release RVFV during blood meal. These findings coupled with the trophic preference for

197 human and domestic animal strengthen the hypothesis of the role of these two mosquitoes'
198 species in the transmission of RVFV in Madagascar although only viral RNA and not
199 infectious viral particles were detected in saliva. This information is critical for implementing
200 vector control to prevent reemergence of RVFV.
201

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210

211 Authors' contributions

212 FNR, JMH and SB coordinate the project and designed experiments. FNR and TNJJN
213 collected and breed mosquitoes and conducted experimental infection of mosquitoes. JPR
214 performed cell culture and virus titration. FNR, TNJJN, SFA, ABF, JMH, SB analyzed the
215 results and wrote the paper. All authors read and approved the final version of the manuscript.

216

217 Competing interests

218 The authors declare that they have no competing interests.

219

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232 **References**

- 233 Andriamandimby, S.F. et al., 2010. Rift valley fever during rainy seasons, Madagascar, 2008
234 and 2009. *Emerging Infectious Diseases*, 16(6), pp.963–970.
- 235 Balenghien, T. et al., 2013. Towards a better understanding of Rift Valley fever epidemiology
236 in the south-west of the Indian Ocean. *Veterinary research*, 44, p.78. Available at:
237 [http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3848763&tool=pmcentrez&r](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3848763&tool=pmcentrez&rendertype=abstract)
238 [endertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3848763&tool=pmcentrez&rendertype=abstract) [Accessed March 23, 2016].
- 239 Bird, B.H. et al., 2007. Highly sensitive and broadly reactive quantitative reverse
240 transcription-PCR assay for high-throughput detection of Rift Valley fever virus. *Journal*
241 *of Clinical Microbiology*, 45(11), pp.3506–3513.
- 242 Chouin-Carneiro, T. et al., 2016. Differential Susceptibilities of *Aedes aegypti* and *Aedes*
243 *albopictus* from the Americas to Zika Virus. *PLoS Neglected Tropical Diseases*, 10(3),
244 pp.1–11. Available at: <http://dx.doi.org/10.1371/journal.pntd.0004543>.
- 245 Clerc, Y. & P, C., 1981. Rapport du Laboratoire des arbovirus 1980. *Arch Inst Pasteur*
246 *Madag.*, 49, pp.65–69.
- 247 Fontenille, D., Mathiot, C. & Coulanges, P., 1988. [Hemorrhagic fever viruses in
248 Madagascar]. *Archives de l'Institut Pasteur de Madagascar*, 54(1), pp.117–24. Available
249 at: <http://www.ncbi.nlm.nih.gov/pubmed/3242423> [Accessed September 7, 2016].
- 250 Fontenille, D., Rakotoarivony, I. & Lepers, J.P., 1989. [Entomological results of the malaria
251 program of the Pasteur Institute in the Malagasy Highland Plateaux in 1987-1988].
252 *Archives de l'Institut Pasteur de Madagascar*, 56(1), pp.275–86. Available at:
253 <http://www.ncbi.nlm.nih.gov/pubmed/2576719> [Accessed July 6, 2016].
- 254 Gray, G.C. et al., 2015. Seroepidemiological Study of Interepidemic Rift Valley Fever Virus
255 Infection Among Persons with Intense Ruminant Exposure in Madagascar and Kenya.
256 *American Journal of Tropical Medicine and Hygiene*, 93(6), pp.1364–1370. Available

257 at: <http://www.ajtmh.org/cgi/doi/10.4269/ajtmh.15-0383> [Accessed September 7, 2016].

258 Kahlon, S.S. et al., 2010. Severe Rift Valley Fever May Present with a Characteristic Clinical
259 Syndrome. *American Journal of Tropical Medicine and Hygiene*, 82(3), pp.371–375.

260 Morvan, J. et al., 1992. First fatal human case of Rift Valley fever in Madagascar.

261 *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 86(3), p.320.

262 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/1412665> [Accessed April 12, 2016].

263 Moutailler, S., Bouloy, M. & Failloux, A.-B.B., 2007. Short report: Efficient oral infection of
264 *Culex pipiens quinquefasciatus* by Rift Valley fever virus using a cotton stick support.

265 *The American journal of tropical medicine and hygiene*, 76(5), pp.827–9. Available at:

266 <http://www.ncbi.nlm.nih.gov/pubmed/17488899> [Accessed April 12, 2016].

267 Nepomichene, T.N.J.J., Elissa, N., et al., 2015. Species Diversity, Abundance, and Host

268 Preferences of Mosquitoes (Diptera: Culicidae) in Two Different Ecotypes of

269 Madagascar With Recent RVFV Transmission. *Journal of Medical Entomology*, 52(5),

270 pp.962–969. Available at: <http://jme.oxfordjournals.org/lookup/doi/10.1093/jme/tjv120>

271 [Accessed September 7, 2016].

272 Nepomichene, T.N.J.J., Tata, E. & Boyer, S., 2015. Malaria case in Madagascar, probable

273 implication of a new vector, *Anopheles coustani*. *Malaria journal*, 14(1), p.475.

274 Available at:

275 [http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4666205&tool=pmcentrez&r](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4666205&tool=pmcentrez&rendertype=abstract)
276 [endertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4666205&tool=pmcentrez&rendertype=abstract).

277 Ratovonjato, J. et al., 2011. Detection, isolation, and genetic characterization of Rift Valley

278 fever virus from *Anopheles* (*Anopheles*) *coustani*, *Anopheles* (*Anopheles*) *squamosus*,

279 and *Culex* (*Culex*) *antennatus* of the Haute Matsiatra region, Madagascar. *Vector borne*

280 *and zoonotic diseases* (Larchmont, N.Y.), 11(6), pp.753–759.

281 Seufi, A.M. & Galal, F.H., 2010. Role of *Culex* and *Anopheles* mosquito species as potential

- 282 vectors of rift valley fever virus in Sudan outbreak, 2007. *BMC Infectious Diseases*,
283 10(1), p.65. Available at: [http://bmcinfectdis.biomedcentral.com/articles/10.1186/1471-](http://bmcinfectdis.biomedcentral.com/articles/10.1186/1471-2334-10-65)
284 2334-10-65.
- 285 Smithburn, K.C., Haddow, A.J. & Gillet, J.D., 1948. Rift Valley fever; isolation of the virus
286 from wild mosquitoes. *British journal of experimental pathology*, 29(2), pp.107–21.
287 Available at:
288 [http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2077096&tool=pmcentrez&r](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2077096&tool=pmcentrez&rendertype=abstract)
289 [endertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2077096&tool=pmcentrez&rendertype=abstract) [Accessed April 12, 2016].
- 290 Tantely, L.M., Boyer, S. & Fontenille, D., 2015. Review article: A review of mosquitoes
291 associated with Rift Valley fever virus in Madagascar. *American Journal of Tropical*
292 *Medicine and Hygiene*, 92(4), pp.722–729.
- 293 Turell, M.J. et al., 2008. Vector competence of selected African mosquito (Diptera: Culicidae)
294 species for Rift Valley fever virus. *Journal of medical entomology*, 45(1), pp.102–108.
- 295 Woods, C.W. et al., 2002. An outbreak of Rift Valley fever in Northeastern Kenya, 1997-98.
296 *Emerging infectious diseases*, 8(2), pp.138–44.
- 297
- 298

299 **Table 1- Infection, dissemination and transmission of RVFV by *Culex antennatus*.**

300

Day post-infection	Infection Rate (IR)	Disseminated Infection Rate (DIR)	Transmission Rate (TR)	Transmission Efficiency (TE)
2	40% (4/10)	0% (0/4)	- (0/0)	0% (0/10)
5	12.5% (3/24)	100% (3/3)	33.3% (1/3)	4.2% (1/24)
8	14.3% (3/21)	100% (3/3)	66.6% (2/3)	9.5% (2/21)
14	33.3% (3/9)	100% (3/3)	100% (3/3)	33.3% (3/9)

301 IR, Infection rate, proportion of infected mosquitoes among tested ones; DIR, Disseminated
302 Infection Rate, proportion of mosquitoes with disseminated infection (head, wings, legs
303 infected) among infected mosquitoes; TR, Transmission Rate, proportion of mosquitoes with
304 infected saliva among mosquitoes with disseminated infection; TE, Transmission Efficiency,
305 proportion of mosquitoes with infected saliva among tested ones.

306

307

308 **Table 2- Infection, dissemination and transmission of RVFV by *Anopheles coustani*.**

Day post-infection	Infection Rate (IR)	Disseminated Infection Rate (DIR)	Transmission Rate (TR)	Transmission Efficiency (TE)
2	15% (3/20)	0% (0/3)	- (0/0)	0% (0/20)
5	15.8% (6/38)	100% (6/6)	83.3 (5/6)	13.2% (5/38)
8	50% (3/6)	100% (3/3)	100 (3/3)	50% (3/6)

309 IR, Infection rate, proportion of infected mosquitoes among tested ones; DIR, Disseminated
310 Infection Rate, proportion of mosquitoes with disseminated infection (head, wings, legs
311 infected) among infected mosquitoes; TR, Transmission Rate, proportion of mosquitoes with
312 infected saliva among mosquitoes with disseminated infection; TE, Transmission Efficiency,
313 proportion of mosquitoes with infected saliva among tested ones.

314

315 **Figure Legend**

316

317 **Fig. 1- The three study sites in Madagascar.** Red dots design the study sites. Engorged and

318 gravid females of *Cx. antennatus* and *An. coustani* were captured in three different sites in

319 Madagascar: in Moramanga (18°51'27.60"S; 48° 7'40.20"E) located in the Alaotra-Mangoro

320 region, in Ankazobe (18°24'16.81"S; 47° 3'4.37"E), in the Analamanga region, and in

321 Tsiroanomandidy (18°50'37.16"S; 46° 1'55.79"E), in the Bongolava region.

322

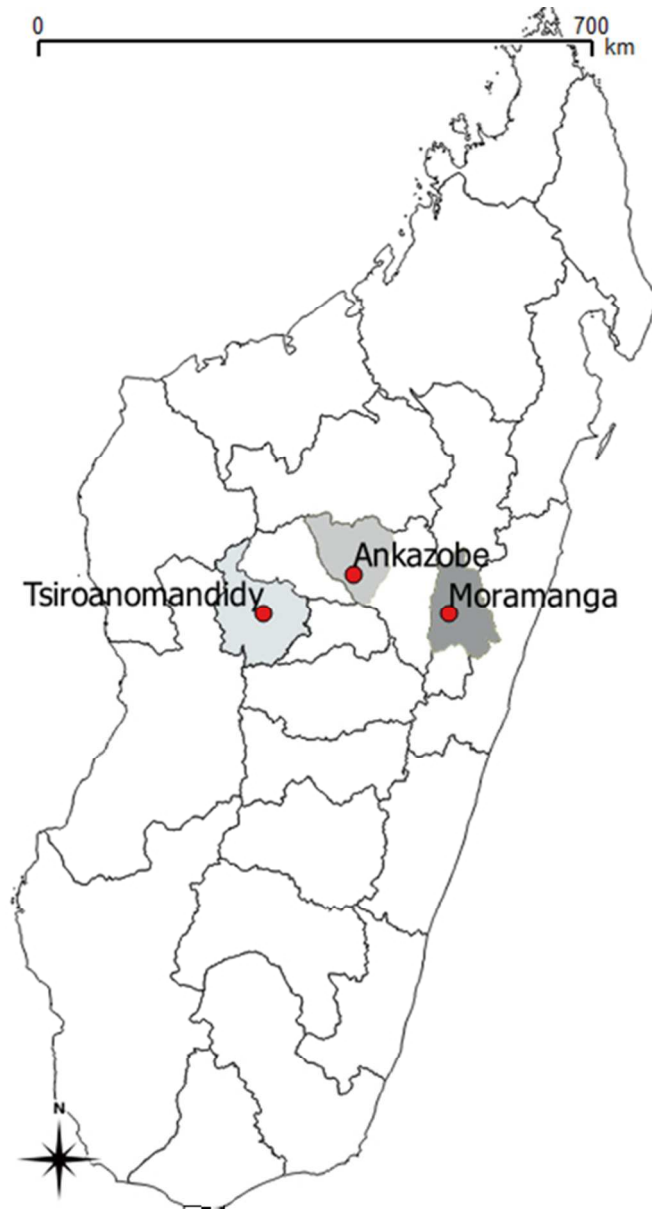


Fig. 1- The three study sites in Madagascar. Red dots design the study sites. Engorged and gravid females of *Cx. antennatus* and *An. coustani* were captured in three different sites in Madagascar: in Moramanga ($18^{\circ}51'27.60''\text{S}$; $48^{\circ}7'40.20''\text{E}$) located in the Alaotra-Mangoro region, in Ankazobe ($18^{\circ}24'16.81''\text{S}$; $47^{\circ}3'4.37''\text{E}$), in the Analamanga region, and in Tsiroanomandidy ($18^{\circ}50'37.16''\text{S}$; $46^{\circ}1'55.79''\text{E}$), in the Bongolava region.

41x73mm (300 x 300 DPI)