**Table S1.** Environmental characteristics of sampled sites.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Regions** | **Landscape** | **Climate** | **Vegetation** | **Coordinates** |
| Bafoussam | Peri-urban | Equatorial moonsoon | Grassland forest | 5,39523N, 10,41744E |
| Buea | Urban | Equatorial mountain moonsoon | Mountain forest | 4,15126N, 9,28343E |
| Edea | Rural | Equatorial mountain moonsoon | Wet dense forest | 3,72715N, 10,12801E |
| Yaoundé | Urban | Equatorial guinean | Wet dense forest | 3,86956N, 11,45550E |

**Table S2.** Primer sequences used in targeting the mitochondrial COI gene in *Aedes* mosquitoes [42].

|  |  |
| --- | --- |
| **Primer** | **Sequences (5′ to 3′)** |
| AU-COI-F | TATTTTCWACAAATCATAARGATATTGGWAC |
| AU-COI-R | TAWACTTCWGGRTGWCCRAARAATCA |

**Table S3.** Primer sequences and probes used in quantifying selected viruses in *Aedes* mosquitoes.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Virus** | | **Primer & Probe** | **Sequences (5′ to 3′)** | |
| Guangzhou  sobemo-like virus | | F | AGCTTTGTTCTCTCGAGGCG | |
| R | ACGTCCTACCAAATTCAGCGA | |
| P | GGCTAAGGAGGAAGATCCTGAGAAAGC | |
| Bafoussam mosquito rhabdovirus  (BMRV) | F | | | AGACGAAACACTCCAGGCAG |
| R | | | AGTAATGGGCAACCGTGAGG |
| P | | | ACGATCAACTACCGTCACTGTCCA |
| Bafoussam mosquito  solemovirus  (BMSV) | F | | | ATCCACGAACCGGTAACACC |
| R | | | ATTCCCTCGAAACACGGCAT |
| P | | | TCCAACGCTGTCGCAACTTCCC |
| Bafoussam mosquito  bunyavirus 1  (BMBV1) | F | | | CTTGTGCCCCTCGATCACTT |
| R | | | AAGAAGAACCAGCACGGAGG |
| P | | | GGTCTCAGCAAAGAGTGCCAGAAT |
| Bafoussam mosquito  bunyavirus 2  (BMBV2) | F | | | TCTGCTGACAATCCCGACAC |
| R | | | GAGCAGCTACGCATTCAAGC |
| P | | | TCACACCTGTGGGCTTTGTTTCTATTT |
| Bafoussam mosquito  orthomyxovirus 1  (BMOV1) | F | | | CCAGGTGGAAAGAACCCTCC |
| R | | | ACCCTGATTTGGGGATGCAG |
| P | | | TCGCAAAGAGGGAGCTTGTGGA |
| Bafoussam mosquito  orthomyxovirus 2  (BMOV2) | F | | | CGAGGGCTCTGTCAATCCTC |
| R | | | TCCCGGAAACATGAGACAGC |
| P | | | CGCACAGCTGGGAGCTCCCA |

**Table S4.** Relative abundance (%) of eukaryotic virus families detected in *Aedes* mosquitoes.

|  |  |  |
| --- | --- | --- |
| **Virus Family** | **Relative Abundance (%)** | |
| *Rhabdoviridae* | | 22.1 |
| *Solemoviridae* | | 19.8 |
| *Orthomyxoviridae* | | 16.3 |
| *Phasmaviridae* | | 5.71 |
| *Totiviridae* | | 5.33 |
| *Chrysoviridae* | | 2.58 |
| *Sedoreoviridae* | | 1.05 |
| *Xinmoviridae* | | 0.408 |
| *Iflaviridae* | | 0.180 |
| *Flaviviridae* | | 0.113 |
| *Partitiviridae* | | 0.0832 |
| *Peribunyaviridae* | | 0.0749 |
| *Anelloviridae* | | 0.0516 |
| *Phenuiviridae* | | 0.0372 |
| *Metaviridae* | | 0.0123 |
| *Circoviridae* | | 0.006 |
| Unclassified viruses | | 26.2 |

**Table S5.** Relative abundance (%) of eukaryotic viruses not classified at family level detected in *Aedes* mosquitoes.

|  |  |
| --- | --- |
| **Unclassified viruses** | **Relative Abundance (%)** |
| Bafoussam mosquito bunyavirus 1 (BMSV1) | 91.1 |
| Kwale mosquito virus | 4.6 |
| Bafoussam mosquito bunyavirus 2 (BMSV2) | 2.5 |
| Hubei toti-like virus 10 | 0.535 |
| Aedes alboannulatus orthomyxo-like virus | 0.374 |
| Hubei mosquito virus 4 | 0.275 |
| San Gabriel mononegavirus | 0.190 |
| Culex luteo-like virus | 0.155 |
| Canya virus | 0.122 |
| Redbank virus | 0.0459 |
| Serbia mononega-like virus 1 | 0.0057 |

**Table S6.** Relative abundance (%) of eukaryotic viruses families detected in *Ae. albopictus* and *Ae. africanus.*

|  |  |  |
| --- | --- | --- |
| **Virus Family** | **Relative Abundance (%)** | |
| ***Ae. albopictus*** | ***Ae. africanus*** |
| *Anelloviridae* | 0.05 | - |
| *Circoviridae* | 0.01 | - |
| *Phasmaviridae* | - | 5.7 |
| *Chrysoviridae* | - | 2.6 |
| *Sedoreoviridae* | - | 1.05 |
| *Xinmoviridae* | - | 0.41 |
| *Iflaviridae* | - | 0.18 |
| *Flaviviridae* | - | 0.11 |
| *Phenuiviridae* | - | 0.04 |
| *Metaviridae* | - | 0.01 |
| *Orthomyxoviridae* | 16.3 | 16.3 |
| *Peribunayviridae* | 0.015 | 0.015 |
| *Solemoviridae* | 0.77 | 0.77 |
| *Totiviridae* | 5.3 | 5.3 |
| Unclassified viruses | 0.07 | 26.1 |

**Table S7.** Relative abundance (%) of eukaryotic viruses families detected in *Ae. albopictus* samples from all locations.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Virus Family** |  | **Relative Abundance (%)** | | |
| **Buea** | | **Edea** | **Yaoundé** |
| *Peribunayviridae* | - | | - | 13.03 |
| *Totiviridae* | - | | - | 13.03 |
| *Orthomyxoviridae* | 6.00 | | - | - |
| *Solemoviridae* | - | | - | 6.00 |
| Unclassified viruses | 6.00 | | - | - |

**Table S8.** Novel viruses identified and their closest known relatives.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Virus taxon** | **Novel Virus**  **(abbreviation)** | **Closest Relative**  **(accession**  **numbers**  **of contigs)** | **GenBank**  **accession numbers** | **Contig Length**  **(bp)** | **Contig**  **Mean BLASTx**  **(%)** | **Proteins** |
| *Bunyavirales* | Bafoussam mosquito bunyavirus 1 (BMBV1) | Salarivirus Mos8CM0  (API61884.1, API61886.1,  API61885.1) | PP764662 | 6602 | 40.60 | RdRp |
| PP764664 | 1246 | 31.98 | S |
| PP764663 | 1881 | 30.57 | M |
| Bafoussam mosquito bunyavirus 2 (BMBV2) | Salarivirus Mos8CM0 | PP868493 | 7712 | 39.02 | RdRp |
| (API61884.1, API61886.1, | PP868495 | 531 | 40.55 | S |
| API61885.1) | PP868494 | 1571 | 32.16 | M |
| *Orthomyxoviridae* | Bafoussam mosquito orthomyxovirus 1 (BMOV1) | Guadeloupe mosquito quaranja-like virus 1  (QRW42591.1, QRW42586.1,  QRW42581.1, QRW42580.1,  QRW42603.1, QRW42599.1,  QRW42610.1) | PP868497 | 2302 | 57.84 | PB1 |
| PP868496 | 2398 | 37.68 | PB2 |
| PP868498 | 2217 | 45.54 | PA |
| PP868499 | 1918 | 39.78 | NP |
| PP868500 | 709 | 47.60 | HP1 |
| PP868501 | 1418 | 36.00 | HP2 |
| PP898301 | 730 | 31.20 | HP3 |
| Bafoussam mosquito orthomyxovirus 2 (BMOV2) | Guadeloupe mosquito quaranja-like virus 1  (QRW42591.1, QRW42587.1,  QRW42581.1, QRW42580.1,  QRW42604.1, QRW42600.1,  QRW42610.1, QRW42611.1) | PP898294 | 2533 | 60.11 | PB1 |
| PP898293 | 2466 | 42.40 | PB2 |
| PP898295 | 2227 | 48.30 | PA |
| PP898296 | 1796 | 45.84 | NP |
| PP898298 | 732 | 62 | HP1 |
| PP898299 | 1357 | 38.57 | HP2 |
| PP898300 | 819 | 37.90 | HP3 |
| PP898302 | 508 | 73.90 | HP4 |
| *Rhabdoviridae* | Bafoussam mosquito rhabdovirus (BMRV) | Ohlsrhavirus  ohlsdorf  (YP\_010086786.1) | PP764659 | 11965 | 55.53 | RdRp  NP  M  G  ORF2  (Protein P?) |
| *Solemoviridae* | Bafoussam mosquito solemovirus (BMSV) | Pyongtaek culex  solemovirus  (UGO57098.1,  UGO57097.1) | PP764660  PP764661 | 2792  1502 | 61.36  47.30 | RdRp  HP  HP  C |

RNA-dependent RNA polymerase (RdRp),Spike protein (S), Matrix protein (M), Nucleoprotein (NP), Plymerase Acidic protein (PA), Polymerase basic protein (PB), Glycoprotein (G), Capsid protein (C), Hypothetical protein (HP).

**Table S9.** Prevalence of selected viruses for qRT-PCR in *Aedes* mosquitoes across all locations.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Virus Name** | ***Aedes* species** | | | | | | |
| **Location** | | | ***Ae. africanus*** | ***Ae. albopictus*** | ***Ae. simpsoni*** | ***Ae. aegypti*** |
| Guangzhou sobemo-like virus | | Bafoussam | 36 – 2.8 – 0.0e+00 | | 10 –10.0 – 0.0e+00 | – | – |
| Buea | – | | 39 – 84.6 – 5.1e+04 | 2 – 100.0 – 2.8e+03 | – |
| Edea | – | | 39 – 20.5 – 0.0e+00 | 5 – 20.0 – 0.0e+00 | 1 – 0.0 – 0.0e+00 |
| Yaounde | – | | 40 – 90.0 – 3.7e+04 | 10 – 70.0 – 1.3e+03 | – |
| Bafoussam mosquito rhabdovirus (BMRV) | | Bafoussam | 36 – 0.0 – 0.0e+00 | | 10 – 0.0 – 0.0e+00 | – | – |
| Buea | – | | 39 – 0.0 – 0.0e+00 | 2 – 0.0 – 0.0e+00 | – |
| Edea | – | | 39 – 12.8 – 0.0e+00 | 5 – 0.0 – 0.0e+00 | 1 – 0.0 – 0.0e+00 |
| Yaounde | – | | 40 – 5.0 – 0.0e+00 | 10 – 10.0 – 0.0e+00 | – |
| Bafoussam mosquito solemovirus (BMSV) | | Bafoussam | 36 – 69.4 – 4.5e+03 | | 10 – 40.0 – 0.0e+00 | – | – |
| Buea | – | | 39 – 2.6 – 0.0e+00 | 2 – 0.0 – 0.0e+00 | – |
| Edea | – | | 39 – 25.6 – 0.0e+00 | 5 – 80.0 – 1.8e+03 | 1 – 0.0 – 0.0e+00 |
| Yaounde | – | | 40 – 10.0 – 0.0e+00 | 10 – 10.0 – 0.0e+00 | – |
| Bafoussam mosquito bunyavirus 1 (BMBV1) | | Bafoussam | 36 – 36.1 – 0.0e+00 | | 10 – 10.0 – 0.0e+00 | – | – |
| Buea | – | | 39 – 0.0 – 0.0e+00 | 2 – 0.0 – 0.0e+00 | – |
| Edea | – | | 39 – 0.0 – 0.0e+00 | 5 – 0.0 – 0.0e+00 | 1 – 0.0 – 0.0e+00 |
| Yaounde | – | | 40 – 0.0 – 0.0e+00 | 10 – 0.0 – 0.0e+00 | – |
| Bafoussam mosquito bunyavirus 2 (BMBV2) | | Bafoussam | 36 – 2.8 – 0.0e+00 | | 10 – 0.0 – 0.0e+00 | – | – |
| Buea | – | | 39 – 10.3 – 0.0e+00 | 2 – 0.0 – 0.0e+00 | – |
| Edea | – | | 39 – 23.1 – 0.0e+00 | 5 – 60.0 – 2.0e+03 | 1 – 0.0 – 0.0e+00 |
| Yaounde | – | | 40 – 0.0 – 0.0e+00 | 10 – 0.0 – 0.0e+00 | – |
| Bafoussam mosquito orthomyxovirus 1 (BMOV1) | | Bafoussam | 36 – 8.3 – 0.0e+00 | | 10 – 0.0 – 0.0e+00 | – | – |
| Buea | – | | 39 – 0.0 – 0.0e+00 | 2 – 0.0 – 0.0e+00 | – |
| Edea | – | | 39 – 0.0 – 0.0e+00 | 5 – 0.0 – 0.0e+00 | 1 – 0.0 – 0.0e+00 |
| Yaounde | – | | 40 – 0.0 – 0.0e+00 | 10 – 0.0 – 0.0e+00 | – |
| Bafoussam mosquito orthomyxovirus 2 (BMOV2) | | Bafoussam | 36 – 25.0 – 0.0e+00 | | 10 – 10.0 – 0.0e+00 | – | – |
| Buea | – | | 39 – 7.7 – 0.0e+00 | 2 – 0.0 – 0.0e+00 | – |
| Edea | – | | 39 – 7.7 – 0.0e+00 | 5 – 0.0 – 0.0e+00 | 1 – 0.0 – 0.0e+00 |
| Yaounde | – | | 40 – 0.0 – 0.0e+00 | 10 – 0.0 – 0.0e+00 | – |

Number of individual *Aedes* tested – Prevalence (%) – Median genome copies.

**Table S10.** Prevalence (%) of selected viruses for qRT-PCR in *Aedes* mosquitoes.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Virus Name** | Prevalence of selected viruses (%) | | | |
| ***Ae. africanus*** | ***Ae. albopictus*** | ***Ae. simpsoni*** | ***Ae. aegypti*** |
| Guangzhou sobemo-like virus | 2.8 | 60.9 | 58.8 | 0 |
| Bafoussam mosquito rhabdovirus | 0.0 | 5.5 | 5.9 | 0 |
| Bafoussam mosquito solemovirus | 69.4 | 14.8 | 29.4 | 0 |
| Bafoussam mosquito bunyavirus 1 | 36.1 | 0.8 | 0 | 0 |
| Bafoussam mosquito bunyavirus 2 | 2.8 | 10.2 | 17.6 | 0 |
| Bafoussam mosquito orthomyxovirus 1 | 8.3 | 0 | 0 | 0 |
| Bafoussam mosquito orthomyxovirus 2 | 25 | 5.5 | 0 | 0 |