Characterization of a Novel Tanay Virus Isolated From Anopheles sinensis Mosquitoes in Yunnan, China

Lu Zhao1,2, Caroline Mwaliko1,2, Evans Atoni1,2, Yujuan Wang1,2, Yunzhi Zhang3, Jianbo Zhan4, Xiaomin Hu1, Han Xia1* and Zhiming Yuan1*

1 Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, China, 2 University of Chinese Academy of Sciences, Beijing, China, 3 Yunnan Institute of Endemic Disease Control and Prevention, Dali, China, 4 Division for Viral Disease with Detection, Hubei Provincial Center for Disease Control and Prevention, Wuhan, China

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INTRODUCTION

Arthropod-borne viruses (arboviruses) cause a significant part of the current global emerging and re-emerging infectious diseases, hence they pose a huge public health risk worldwide. The global spread, emergence and re-emergence of these arboviruses has been promoted by several factors including increased global trade (Cleton et al., 2012), diverse and changing environmental conditions (Soverow et al., 2009; Morin and Comrie, 2013) and the wide geographical distribution range of mosquitoes (Weaver and Reisen, 2010). Different arthropods including mosquitoes are known to spread arboviruses via complex replicative lifecycle in both the arthropod vector and vertebrate hosts (dual-host) (Vasilakis and Tesh, 2015; Halbach et al., 2017). Despite causing arbovirus infections, mosquitoes are also known to harbor a wide array of other viruses.
Culicidae (Shi et al., 2016); commonly referred to as insect-specific viruses (ISVs) that persistently infect insects (single-host) and only replicate on insect cell lines. Their inability to infect mammalian hosts might be as a result of their heat sensitivity (Aliota et al., 2012; Marklewitz et al., 2015), lack of cell surface receptors and host factors (Nasar et al., 2015; Halbach et al., 2017; Hermanns et al., 2017). Because of their inability to cause diseases in humans and their far less or even absent economic impact on animals and plants, ISVs are largely neglected and very few have been identified and characterized in the past. However, modern-day advances in deep sequencing technology and bioinformatic tools have paved a way for the effective discovery of mosquito viruses. Consequently, many ISVs have been described from different mosquito species and majority of these newly discovered ISV’s phylogenetically cluster to similar viral groups associated with classical arboviruses. They include Flavivirus (Crabtree et al., 2003; Sang et al., 2003; Cook et al., 2006; Zuo et al., 2014; McLean et al., 2015), Alphavirus (Nasar et al., 2012), Bunyavirus (Marklewitz et al., 2011, 2013; Auguste et al., 2014), Mononegavirus (Vasilakis et al., 2014; Wang et al., 2017), Almendravirus (Contreras et al., 2017), Reovirus (Attooi et al., 2005; Hermanns et al., 2014; Auguste et al., 2015; Harrison et al., 2016), and Negevirus (Vasilakis et al., 2013; Auguste et al., 2014; Kallies et al., 2014; Nabeshima et al., 2014; Carapeta et al., 2015; Kawakami et al., 2014; Hang et al., 2016; Kawakami et al., 2016; Fujita et al., 2017; O’Brien et al., 2017; Moraes et al., 2019; Wang et al., 2019; Zhou et al., 2019). Moreover, they are thought to be the ancestors of arboviruses (Marklewitz et al., 2015). Possible application of these insect-specific viruses in vaccine development and use as biocontrol agents have been investigated (Bolling et al., 2015). It has been hypothesized that ISVs might act as natural regulators of arboviral infection, replication, and transmission (Hobson-Peters et al., 2013; Hall et al., 2016; Hall-Mendelin et al., 2016). Therefore, their high abundance, extensive viral taxa distribution and association to classical arboviruses warrants more in-depth research.

Negevirus is a new taxon of ISVs first described by Vasilakis’ team (Vasilakis et al., 2013). They are spherical or elliptical in shape with particle size of 45–55 nm in diameter. Their genomic RNA is non-segmented, polyadenylated positive sense strand with a genome length ranging from 9 to 10 kb. In addition, they have limited untranslated regions (UTRs) containing three major open reading frames (ORFs). Some of the viruses in this taxon including Tanay virus (TANAV) (Nabeshima et al., 2014), Castlerkea Virus (O’Brien et al., 2017) and Okushiri virus (Kawakami et al., 2016) have projection-like structures. This taxon has two groups, namely Sandewavirus and Neloripivirus, which are distantly related to some plant viruses, such as Cileviruses, Higeivirus, and Blunervirus (Quito-Avila et al., 2013; Carapeta et al., 2015). Notably, negeviruses have been described to infect a wide range of hematophagous insects including 9 mosquito genera from the Culicidae Family: Culex spp., Aedes spp., Anopheles spp., Armigeres spp., Psorophora spp., Uranotaenia spp., Deinocerites spp., Wyeomyia spp. and Trichoprosom spp. (Nunes et al., 2017). Geographically, negeviruses have been described in Asia (Vasilakis et al., 2013; Nabeshima et al., 2014; Hang et al., 2016; Kawakami et al., 2016; Fujita et al., 2017; Nunes et al., 2017; Wang et al., 2018; Zhao et al., 2018), Africa (Vasilakis et al., 2013; Kallies et al., 2014), Oceania (O’Brien et al., 2017), Europe (Carapeta et al., 2015), South America (Vasilakis et al., 2013; Nunes et al., 2017; Moraes et al., 2019), Central America (Auguste et al., 2014; Nunes et al., 2017), and North America (Vasilakis et al., 2013; Nunes et al., 2017; Charles et al., 2018), implying their wide global distribution. Downstream, experimental studies have demonstrated the ability of negeviruses to replicate and co-infect host cells with other viruses. For instance, a study conducted by Vasilakis et al. (2013) on adult Ae. aegypti and Ae. albopictus mosquitoes with Negevirus established that the virus was more likely transmitted via vertical or transovarial transmission rather than oral infection, relying on high viral titer among their insect hosts. In addition, Carapeta et al. (2015) reported that co-infection of Negevirus-OCNV virus forms an intermediate dsRNA, which could trigger a RNA silencing mechanism, by delivering dsRNA targeting the “lethal” gene transcript to the intended pathogen, this mechanism can knock down this gene and lead to insect mortality revealing that negeviruses hold great potential that can be applied in “species-specific” insect biological control strategies (Vogel et al., 2018). Thus, more information is required to understand the virus-host interaction in nature (Quito-Avila et al., 2013; Carapeta et al., 2015). Moreover, multiple hairpin structures have been predicted in both 5′ and 3′ UTRs, especially in the 5′-UTR region, there is an IRES (Internal Ribosome Entry Site) structure that allows for cap-dependent translation of the viral RNA (Gorchakov et al., 2014). Mutating the IRES element or adding an inhibitor, reduces the ability of the virus to replicate to produce attenuated strains (Pestova et al., 1996; Li et al., 2004). Therefore, negeviruses can be developed as a potential vehicle for vaccine production.

Tanay virus was first isolated in the Philippines from pools of Culex spp. and Armigeres spp., in 2013 (Nabeshima et al., 2014). In the same year, other strains of TANAV were isolated from Culex tritaeniorhynchus and C. quinquefasciatus mosquitoes in Guangxi, China (Wang et al., 2018). However, these two studies only described preliminarily findings on TANAV viral morphology, genome organization, and phylogeny. In the surveillance program of vector-borne viruses in mosquitoes in Yunnan, China, TANAV strain YN15_103_01 was isolated in our laboratory from Anopheles sinensis. Here, we describe its extensive characterization, including the viral structure, growth curve in mosquito and vertebrate cells, genome orientation, coding proteins and phylogeny. Our findings are important and crucial for a better understanding of TANAV properties and their effect on persistence infection in mosquitoes and arbovirus transmission.

MATERIALS AND METHODS

Mosquito Collection and Sample Preparation
Adult Anopheles sinensis mosquitoes were trapped from Dehong, Lincang, Pu’er, Xishuangbanna and Honghe, Yunnan province, China, cities bordered Myanmar, Laos and Vietnam. Sampling was conducted between July and September, 2015. A total of
Mosquito macerates were clarified by centrifugation at 20,000 g for 2 hours, in a Type 70 Ti rotor (Beckman). Upon completion, the formed virus fractions were carefully aspirated out while the sucrose solution was discarded. Virus purification was then conducted through loading of the obtained virus fractions onto a new ultracentrifuge tube containing PBS and 2% penicillin/streptomycin. Cells were incubated at 28°C in 5% CO₂ for 3 days to allow plaque formation. The overlay was removed and monolayers were fixed with 750 μl of 10% formaldehyde in PBS for 30 min. The cells were then stained with 2% crystal violet in 30% methanol for 5 min at room temperature; excess stain was removed under running water, and plaques were counted and recorded as per the plaque forming assay (measured in PFU).

**Virus Replication Kinetics**

Virus replication kinetic studies were undertaken to examine the host range of the virus. Two mosquito cell lines namely, C6/36 and Aag2, and three mammalian cell lines namely, Vero, BHK-21, and SW13, were plated in a 25-cm² culture flask (2 x 10⁶ cells). C6/36 cells were inoculated with virus at MOIs of 1, 0.01, and 0.0001 PFU/cell, while Vero, BHK-21 and SW13 cells were inoculated with virus at MOIs of 1, 0.01 PFU/cell. Aag2 and C6/36 cells were inoculated with virus at MOIs of 1, 0.01 PFU/cell, which was performed to compare the viral replication in two different mosquito cells. The cell-free supernatants of the infected cell lines were collected at periodic intervals, and each aliquot was tittered by plaque assay on C6/36 cells (Kuwata et al., 2013).

**Nucleic Acid Extraction and Genome Sequencing**

To determine the full genome sequence of the viral isolate, total RNA was extracted using RNeasy mini kit (Qiagen, Germany) and strand-specific libraries were prepared using the TruSeq® Stranded Total RNA Sample Preparation kit (Illumina, United States) following the manufacturer’s instructions. Sequencing was performed at Shanghai Biotechnology Corporation using the Illumina HiSeq 2, 500 (Illumina, United States) platform. Generated sequence reads were de novo assembled using Trinity in Galaxy platform (Göertz et al., 2019).

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1. http://mississippi.snv.jussieu.fr
Protein Analysis
The purified viral particles were lysed directly in a water-bath with 5 × SDS loading buffer [250 mM Tris-HCl, pH 6.8, 10% SDS (W/V), 0.5% bromophenol blue (W/V), 50% glycerol (W/V), 5% β-mercaptoethanol (W/V)] for 10 min at 100°C. Subsequently, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% polyacrylamide gel was employed to separate the viral protein bands, with the gel being stained with Fast Silver Stain using Protein Stains O (Sangon, China). Putative opening reading frame (ORF) organization was predicted by the Softberry program (Shahmuradov et al., 2017)\(^3\). Conserved domains were predicted by HHpred (Söding et al., 2005)\(^5\) and BLAST search on the National Center for Biotechnology Information (NCBI) database. Prediction of trans-membrane regions was performed using TMpred (Hofmann, 1993)\(^4\) and TMHMMv2.0 (Krogh et al., 2001)\(^6\). Visualization of transmembrane domains (TMDs) by Protter (Omasits et al., 2014)\(^6\).

Phylogenetic Analysis
For identification and evolutionary analyses, ORF1 amino acid (aa) sequences of the negeviruses and all members of the family Virgaviridae were aligned using Clustal Omega (Harte et al., 2004)\(^7\). Ambiguously aligned regions were removed and the methyltransferase, helicase and the RdRp conserved protein domains in ORF1 were connected without a gap (Kallies et al., 2014) by using BioEdit Sequence Alignment Editor. Phylogenetic trees were inferred in maximum likelihood/rapid bootstrapping run and 1000 bootstrap replications on XSEDE [RAxML-HPC2 on XSEDE (8.2.10)\(^3\)]. The evolutionary tree was visualized and edited by Figtree and 1000 bootstrap replications.

RESULTS

Virus Isolation, Morphology and Sequencing
In the 17 homogenized samples of Anopheles sinensis mosquito pools, one caused exuberant CPE 48 h.p.i. after inoculation into the C6/36 cells, and the purified virion was one ellipsoid (about 40 × 60 nm) with a projection-like structure (16–20 nm) observed by the electron microscope (Figure 1A). Other than this, the viral particle was able to form large plaques with an unclear borderline in monolayers of C6/36 cells (Figure 1B). Thereafter, we acquired its full genome sequence through next generation sequencing. BLASTn analysis showed the sequence was closely related to TANAV isolate that was first reported from the Philippines (Nabeshima et al., 2014) and Guangxi, China (Wang et al., 2018). Hence, the viral isolate was named TANAV isolate YN15_103_01, since it was isolated from pool number #103. TANAV isolate YN15_103_01 complete genome sequence was assigned to GenBank under accession number MG673930, and the information of the sequence was published in the Genome announcement (Zhao et al., 2018).

Virus Ultrastructural Characteristics
At 48 h.p.i. infected C6/36 cells exhibited clear and obvious CPE as most of them were rounded and ruptured (Supplementary Figure S1). Electron microscopy analysis revealed that the state of core organelles inside the C6/36 cells were enormously different in TANAV isolate YN15_103_01 infected C6/36 cells (Figures 2B–G), compared to the mock-uninfected cells (Figure 2A). The Perinuclear Space (PNS) of vesicles was dilated (Figure 2B), in line with previously described ultrastructural characteristic of negeviruses (Vasilakis et al., 2013; Auguste et al., 2014; Carapeta et al., 2015). In addition, the rough endoplasmic reticulum was deformed, expanding and extending from the nuclear membrane and to the cell membrane (Figures 2B–D) and filled with Para crystalline arrays (Figure 2B) and microtubules (Figure 2E). All of the above changes in organelles’ structures could be viral-exploited effects necessary for the proteins required for assembly of the virus, while the final virion maturation takes place in the cytoplasm (Figure 2G). Further, the formation of cytoplasmic cytopathic vacuoles (CPVs) (Figure 2F) and autolysosomes (Figure 2G) are the most prominent reasons for the observed CPE in the infected cells.

Virus Replication
TANAV isolate YN15_103_01-infected C6/36 cell lines produced extensive CPE at 24 h.p.i. 48 h.p.i. and 72 h.p.i. as MOI decreased from 1 to 0.01 and 0.0001 (Figure 3A). However, no overt cytopathic effects were observed in Aag2 cell line and three vertebrate cell lines up to 7 days post-infection p.i. (data...
with 20 aa N-terminal signal peptide, according to the protter and is predicted to contain four transmembrane regions domain (RdRp) at nt 5268 to 6588. ORF2 starts at nt 6749 to 8663–9310 nt, which edited putative virion membrane protein (Figure 4A). To further analyze the major structural proteins of TANAV, proteins bands were observed. Six of the bands were corresponding to the predicted ORF2 (594 aa), RdRp (440 aa), Hel (418 aa), vMet (349 aa), FtsJ (200 aa), M (124 aa), respectively. Further, we also found an approximately 100-kDa protein from virus. However, its size is not consistent with the prediction from the full-length ORF sequences (marked as*) (Figure 4C). The genomic RNAs included three open reading frames ORF1, ORF2, and ORF3, and the amino acid sequence similarity among the strains of TANAV was 89.75–95.12%, 73.55–88.22%, and 92.09–96.28%, respectively.

Virus Genome and Protein Analysis

TANAV isolate YN15_103_01 is a 9, 587 nt single-stranded, positive sense virus with poly (A) tail. BLASTn analysis show it’s full genome shares a 79.02% nucleotide sequence identity to the TANAV that was isolated in Philippines (Nabeshima et al., 2014), and a 85.53% nucleotide sequence identity to the TANAV that was isolated from Guangxi, China (Wang et al., 2018). There are three Putative open reading frames (ORFs); ORF1, ORF2 and ORF3. They are flanked by UTRs, while each ORF is separated by two short intergenic regions. ORF1 starts at nt 78 to 6725, and it contains four putative functional protein domains: (i) a viral methyltransferase (vMet) at nt 228 to 1275; (ii) an RNA ribosomal methyltransferase (FtsJ) at nt 2241 to 2841; (iii) a Helicase (Hel) at nt 3483 to 4737; and (iv) an RNA-dependent RNA polymerase domain (RdRp) at nt 5268 to 6588. ORF2 starts at nt 6749 to 8533 and is predicted to contain four transmembrane regions with 20 aa N-terminal signal peptide, according to the protter software (Figures 4A,B). This result is consistent with the other reported strains of TANAV (data not shown). ORF3 ranges from 8663–9310 nt, which edited putative virion membrane protein (Figure 4A). To further analyze the major structural proteins of TANAV, proteins bands were observed. Six of the bands were corresponding to the predicted ORF2 (594 aa), RdRp (440 aa), Hel (418 aa), vMet (349 aa), FtsJ (200 aa), M (124 aa), respectively. Further, we also found an approximately 100-kDa protein from virus. However, its size is not consistent with the prediction from the full-length ORF sequences (marked as*) (Figure 4C). The genomic RNAs included three open reading frames ORF1, ORF2, and ORF3, and the amino acid sequence similarity among the strains of TANAV was 89.75–95.12%, 73.55–88.22%, and 92.09–96.28%, respectively.

Phylogenetic Analysis

Unrooted gap-free phylogenetic trees were constructed based on concatenated amino acid sequences of fused methyl transferase, viral helicase and RdRp domains. The results indicate that TANAV strains could be clearly divided into two small groups; TANAV isolate YN15_103_01 laid on a small independent branch but closer to Guangxi strains than from the one from Philippines. All of them belong to the subclade Sandewavirus in Negevirus (Figure 5A). Following discovery of more Negevirus species, the two clades tentatively named Nelorpivirus and Sandewavirus are more likely to be proven to form taxonomic groups on genus level from their respective evolutionary relationships and phylogenetic distances (Figure 5B).

Other than this, the topology tree of Negevirus with plant-infecting viruses of the genera Cilevirus, Higrevirus and Blunervirus was debatable. Here, we found the out-group viral family Virgaviridae formed a solitary branch, and genera Cilevirus, Higrevirus, and Blinervirus the closest but still
distant relatives formed the other branch with Negevirus, which was more closely genetically related to Nelorpivirus than Sandewavirus in previous reports (Nunes et al., 2017). However, the topology that Nelorpivirus was more closely to Sandevavirus than genera Cilevirus, Higrevirus, and Blinervirus was more likely in the light of similar genome organizations and hosts cluster (Kallies et al., 2014).

DISCUSSION

In this study, we isolated and characterized Tanay virus-isolate YN15_103_01, from Anopheles sinensis mosquitoes collected in Yunnan province, China. Our isolate's nucleotide identity was 79.02% similar to the TANAV that was reported in Philippines (Nabeshima et al., 2014), and 85.53% identical to the TANAV reported from Guangxi, China (Wang et al., 2018). TANAV phylogenetically clusters under genus Negevirus (Figure 5): a new taxon that contains insect specific viruses (ISVs) including Negev virus, Goutanap virus, Bustos virus, Santana virus, Uxmal virus and Manglia virus, among others. Morphologically, the viral particle of TANAV is distinctive with a projection-like structure (Figure 1A). This finding is in line with previously conducted studies (Nabeshima et al., 2014). However, we did not identify the projection-like structure of the virus particles in the cell sections and know when it was assembled. This can be due to: (1) we did not choose good timing and angle of the slice or; (2) perhaps the tail is packaged when it is budding from the virus or it was a; (3) limitation of the used microscope. The recently developed cryo-EM, that has less artifact-prone alternative to thin-section, might be the best instrument of observation in this case (Serwer et al., 2018). Besides, the TANAV virus projection-like structure is comparable to the injection needle of phages (Guan et al., 2019), hence future studies should investigate whether the structure plays a role in virus binding to the host cell receptors, increasing its adsorption during virus entry. Such studies may employ use of reverse genetics to generate a mutant viral strain that lacks the projection-like structure.

Tanay virus did not replicate in the vertebrate cell lines tested, implying it is not an infectious agent in vertebrates. Steady TANAV growth and high titers were observed in Aag2 cells but with no CPE observed up to 7 days pi. compared to C6/36 cells that exhibited extensive CPE after 48 h.p.i. The non-cytopathic growth of TANAV in Aag2 cells might be due to establishment of a latent infection by the virus in Ae. aegypti mosquitoes. Unlike C6/36 cell lines that are Dicer-2 deficient, Aag2 cell lines are able to produce viral siRNAs, in turn the siRNA response suppresses viral replication processes in the Ae. aegypti cells to low tolerant levels in the host cell, thus supporting a lasting infection with no or minimal cytopathic effects (Göertz et al., 2019). Hence, Ae. aegypti mosquitoes might be natural reservoir hosts of TANAV in natural environment habitat. Further, TANAV may be infecting several mosquito species in nature since past reported studies, including ours, have described isolation of TANAV virus from Culex, Armigeres and Anopheles mosquitoes. However, recently described Uxmal virus was found not to

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**FIGURE 3** Replication of TANAV isolate YN15_103_01 in cells. (A–C) The growth curve of TANV isolate YN15_103_01 (MOI = 0.1–0.0001) was measured by Plaque assay at 6–168 h post infection.
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**FIGURE 4** | Bioinformatic and physical analysis of TANAV isolate YN15_103_01 structural proteins. (A) Three open reading frames (ORFs) were predicted in full genome, ORF1 encoded viral methyltransferase (vMet), RNA ribosomal methyltransferase (FtsJ), Helicase Helicase (Hel), RNA dependent RNA polymerase (RdRp). ORF2 contained transmembrane regions, ORF3 encoded membrane protein (M). (B) N-terminal signal peptide (S) and a C-terminal transmembrane domain (TM) of ORF2 predicted by protter. This protein contains 4 transmembrane (TM) regions. (C) Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis performed on purified virions shows 6 abundant proteins corresponding to the predicted ORF2 (594 aa), RdRp (440 aa), Hel (418 aa), vMet (349 aa), FtsJ (200 aa), M (124 aa), respectively. * means no predicted functional protein was corresponding.

Replicate in *Anopheles* mosquito cells, suggesting its insect host range is restricted (Charles et al., 2018). Therefore, it is essential to study the host range, virulence, and tissue tropism of negeviruses across different mosquito species. This information will help to elucidate how negeviruses (including TANAV) are maintained in nature. In addition, *in vivo* and *in vitro* co-infection of TANAV with other representative strains of classical arboviruses should be carried out in mosquitoes and mosquito cell lines to detect whether it has any effect on infectivity and virulence of these arboviruses.

The TANAV genome architecture comprised of three putative open reading frames (ORFs): ORF1, ORF2 and ORF3 located at positions 78 to 6725 nt, 6749 to 8533 nt and 8, 663–9, 310 nt, respectively. The hypothetical ORF3 of negeviruses share a conserved sequence similar to the p24 protein of CiLV-C (YP_654543) (Nabeshima et al., 2014). Specifically, ORF1 comprised of four putative functional protein domains namely viral methyltransferase (vMet), RNA ribosomal methyltransferase (FtsJ), Helicase (Hel) and RNA-dependent RNA polymerase domain (RdRp) (**Figure 4**). ORF1 protein Helicase function contained a conserved tobacco mosaic virus replicase subunit alpha/beta domain (3VKW_A). Although little is known about the associations between negeviruses and plant viruses, these observations raise the possibility of cross-kingdom virus transfer between insects and plants in ancient times. In further characterization of Endogenous Viral Element (EVE) candidates derived from virga/nege-like viruses in insect genomes, within a clade of the insect tobacco-like group (containing a conserved tobacco mosaic virus (TMV)-coat superfamily domain (accession CL20208), obtained data strongly supports the view that insect EVE candidates related to virga/nege-like viruses may be the footprints of ancient insect RNA viruses, but not plant RNA viruses (Kondo et al., 2019), and might indicate there would be a larger taxonomic group including plant viruses and negeviruses. Hence, we hypothesize that if negeviruses evolved from plant viruses, then they ought to have undergone a two-host adaptation phase. Therefore, there must be some viruses that can replicate in plants and insect cells. To ascertain this hypothesis, studies that test growth of negeviruses on plant cells and/or plant viruses (like cileviruses,
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higreviruses and blunerviruses) growth on insect cells should be designed and carried out.

The continuous rise in discovery of ISVs, including negeviruses, has presented a promising and exciting field of study in mosquito-virus biology. So far, about 22 negevirus species have been described worldwide (Figure 6). These negeviruses appear to be limited to latitudes 42°N and 42°S; which falls on tropical and sub-tropical temperate regions, indicating that their distribution could be influenced significantly by environmental and climatic factors. Therefore, new insights on negeviruses and related ISVs, including TANAV, will offer far reaching important and profound knowledge not only to the biological impact on

FIGURE 5 | Analysis of a gap-free concatenated alignment of fused methyltransferase, viral helicase and RdRp domains of members of negeviruses, cileviruses, higreviruses and blunerviruses, as well as representative members of each genus of the Virgaviridae family. A: ML-Tree; B: SDT. TANAV isolate YN15_103_01 (red font); negeviruses (green font). Data shown on Supplementary File S1.

FIGURE 6 | World map showing the global distribution of negeviruses. Data shown on Supplementary File S1. (A–C) indicates a partially enlarged area and numbers and sorts them from left to right.
mosquito populations but also to ongoing translational studies that aim to manipulate mosquito specific viruses as biological agents and vaccines against classical arboviruses that are more common in temperate regions, worldwide.

DATA AVAILABILITY

The datasets generated for this study can be found in the TANAV isolate YN15_103_01 complete genome sequence was assigned to GenBank under accession number MG673930.

AUTHOR CONTRIBUTIONS

ZY, HX, and LZ designed the experiments. LZ and YW performed the experiments. LZ, CM, and HX analyzed the data. ZY, HX, YZ, and JZ contributed reagents, materials, and analysis tools. LZ, CM, EA, XH, HX, and ZY wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.01963/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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