Virome profiling of rodents in Xinjiang Uygur Autonomous Region, China: Isolation and characterization of a new strain of Wenzhou virus

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ABSTRACT

Rodents, as the most diverse and widest distributed mammals, are a natural reservoir of many zoonotic viruses. However, little is known about the viral diversity harbored by rodents in China. Here we performed viral metagenomic analyses of 314 wild rodents covering 7 species, sampled in North-western China. We also conducted a systematic virological characterization of a new Wenzhou virus (WENV) isolate, QARn1, from a brown rat (*Rattus norvegicus*). Full genomic and phylogenetic analyses showed that QARn1 is a previously unidentified strain of *Wenzhou mammarenavirus* and forms a new branch within the Asian clade. Experimental infection of Sprague-Dawley rats with QARn1 did not present overt pathology, but specific humoral immune responses developed and mild hemorrhage and immunocyte infiltration of the lungs and thymus were observed. These observations have expanded the geographic distribution of WENV to Central Asia, and further confirm that brown rats are natural hosts of Wenzhou virus.

1. Introduction

Emerging infectious diseases, which exert global threats to public health and economies, are predominantly zoonoses, with over 70% estimated to originate in wildlife (Jones et al., 2008). As the most abundant, diverse, and geographically distributed mammals, rodents (order Rodentia) are important reservoir hosts of various zoonotic viral pathogens, exemplified by orthohantaviruses and mammarenaviruses (Bordes et al., 2015; Mills, 2006). The Xinjiang Uygur Autonomous Region (abbreviated to Xinjiang), located in North-western China, provides an important terrestrial corridor of the Silk Road connecting China with Central/Western Asia and Europe. This vast region, with a temperate continental climate, consists mostly of desert and grassland, the latter supporting well developed animal husbandry and agricultural industries as well as an ecosystem of rodents belonging to 86 species (Guzalnur·Z et al., 2014). Xinjiang is also the epidemic focus of diverse zoonoses, including Crimean-Congo hemorrhagic fever and plague, caused by agents transmitted by ticks (Guo et al., 2017; Sun et al., 2009;

Yen et al., 1985; Zhang et al., 2004) and the latter caused by *Yersinia pestis* (Li et al., 2009). These zoonotic diseases occasionally lead to outbreaks in humans and profoundly burden the available resources for their control and containment (Wolfe et al., 2007). Viruses carried by rodents in this vast region have been poorly investigated, although they potentially pose a major risk to public health, particularly to agricultural workers in direct or indirect contact with infected animals.

Arenaviruses comprise a large group of enveloped viruses within the family *Arenaviridae* that feature bi-segmented ambisense single-stranded (ss) RNA genomes, while tri-segmented arenaviruses have recently been described in fish (Shi et al., 2018). There are currently 3 genera, *Mammarenavirus*, *Reptarenavirus* and *Hartmanivirus* with, respectively, 35, 5 and 1 recognized members (Maes et al., 2018; Radoshitzky et al., 2015). Mammarenaviruses can cause asymptomatic infections of rodents, and infected animals move freely in their natural habitats while constantly excreting virus into the environment. Humans coming into contact with these agents may develop severe disease (depending on the infecting viruses) such as hemorrhagic fever with up

to 30% case fatality rates, as in Lassa fever in Western Africa caused by Lassa virus (LASV) (McCormick et al., 1987), and Argentinian hemorrhagic fever in South America caused by Junín virus (JUNV) (Peters, 2002). Mammarenaviruses are comprised of two complexes based on serologic, genetic, and geographic relationships (Buchmeier et al., 2013; Radoshitzky et al., 2015): the New World (NW) or Tacaribe complex and the Old World (OW) or Lassa-lymphocytic choriomeningitis complex. The NW complex contains 4 lineages, many members of which are associated with fatal hemorrhagic disease in humans, such as JUNV, Machupo virus (MACV) and Guanarito virus (GTOV), while the OW complex currently consists of a single lineage with 17 species distributed in Africa and Eurasia (Jamieson et al., 2006). Compared to the situation in Africa and the Americas, there is very limited data available regarding mammarenaviruses in Asia. Besides the ubiquitous lymphocytic choriomeningitis virus (LCMV) reported in this area (Morita et al., 1996), Wenzhou virus (WENV) has recently been detected in brown rats (*Rattus norvegicus*), Pacific rats (*R. exulans*), yellow-breasted rats (*R. flavipectus*), black rats (*R. rattus*), lesser ricefield rats (*R. losea*) and white-bellied rats (*Niviventer niviventer*) and one shrew species, the Asian house shrew (*Suncus murinus*), in South China and South-eastern Asia (Blasdell et al., 2016; Li et al., 2015b; Liu et al., 2017; Zhang et al., 2018). In addition, Loei River virus (LORV), another Asian mammarenavirus, has been identified in bandicoot rats (*Bandicota* sp.). Ryukyu virus (RYKV) is the third Asian mammarenavirus, found recently in Yunnan Province, south China, and carried by the Ryukyu mouse (*Mus caroli*) (Wu et al., 2018c). These three viruses have recently been approved as new species by the International Committee on Taxonomy of Viruses (ICTV), and named *Wenzhou mammarenavirus*, *Loei River mammarenavirus* and *Ryukyu mammarenavirus* respectively (Adams et al., 2017; Blasdell et al., 2016; Maes et al., 2018).

To better understand the diversity of viruses harbored by rodents in Asia, the present study was conducted in Xinjiang where rodents were captured and a viral metagenomic analysis of rodent tissues was performed. Results revealed viral sequences annotated to 24 virus families, including known pathogens of mammals and arthropods such as parvoviruses, dicistroviruses, bunyaviruses, paramyxoviruses and arenaviruses. We further conducted systematic virological characterization of a newly isolated arenavirus, QARn1, a strain of WENV isolated in Qapqal (Chábùcháěr) Xibe Autonomous County, Ili Kazakh Prefecture in northern Xinjiang, thereby expanding the already wide geographic distribution of this group of viruses. Animal infection, pathological inspection and interspecies transmission assessment have indicated that *R. norvegicus* is the natural host of the new isolate.

2. Results

2.1. Viromic profiling of rodents

During the period 2012–2016, to investigate viral pathogens harbored by rodents in North-western China, a total of 314 rodents were collected from pasture habitats in 5 Xinjiang locations (Jinghe, Alashankou, Yining, Qapqal and Aksu). Organs (kidneys, lungs, livers and rectums) were removed and transported on dry ice to the laboratory for further characterization (Fig. 1A). The sampling covered 7 rodent species (Fig. 1B) of 7 genera within 3 families: Ural field mouse (*Apodemus uralensis*) (n = 16), brown rat (*R. norvegicus*) (n = 51), great gerbil (*Rhombomys opimus*) (n = 46), Libyan jird (*Meriones libycus*) (n = 50) and house mouse (*Mus musculus*) (n = 1) within the family *Muridae*; long-tailed ground squirrel (*Urocitellus undulates*) (n = 100) within the family *Sciuridae*, and common vole (*Microtus arvalis*) (n = 50) within the family *Cricetidae*.

Samples of lung and rectum from different rodents and locations were pooled into 3 groups (Group AJ: samples from Alashankou and Jinghe; Group YQ: Yining and Qapqal; Group AK: Aksu) and labeled using different barcodes before being subjected to viral metagenomic

analysis as per our previously published method (He et al., 2013). After removal of host-derived sequences, 5,156,956 reads were obtained with an average length of 170 nt, with 7067 (0.14%) being annotated to viruses of 24 families and some unclassified viruses, including dsDNA, dsRNA, ssDNA and ssRNA viruses of mammalian, plant, insect, and bacterial origin. As shown in Fig. 2, the dsDNA viruses were predominantly bacteriophages such as myoviruses, podoviruses, and siphoviruses. The ssDNA viruses comprised two mammal-infecting families, *Anelloviridae* (genus *Alphatorquevirus*) and *Parvoviridae* (genera *Bocaparvovirus* and *Dependoparvovirus,* as well as another unclassified parvovirus further identified as a chapparvovirus). A single unclassified dsRNA partitivirus was identified. The ssRNA viruses were divided into positive sense [ssRNA(+)], negative sense [ssRNA(-)] and reverse transcription [ssRNA (RT)] viruses. The ssRNA(+) group consisted of insect-infecting viruses of 6 viral families (including *Dicistroviridae* and *Iflaviridae*), mammalian ssRNA(-) virus isolates belonging to the families *Arenaviridae* and *Paramyxoviridae*, and insect ssRNA(-) virus isolates belonging to the families *Peribunyaviridae* and *Nairoviridae*, while ssRNA(RT) isolates were composed of members of the *Retroviridae* that could be further classified into 7 genera, such as *Betaretrovirus*, *Deltaretrovirus*, *Epsilonretrovirus*, and *Spumavirus,* together with others remaining unclassified.

The virus-like reads of mammal- or arthropod-infecting ssDNA (parvovirus), ssRNA(+) (dicistrovirus) and ssRNA(-) (paramyxovirus, orthonairovirus and peribunyavirus) were selected and assembled into contigs based on overlapping sequences (Table S1). The contigs were mapped against their reference sequences exhibiting the highest nt identities (Fig. 3). The longest (> 200 nt) or most conserved contigs were subsequently employed for phylogenetic analyses and calculation of genetic distances to most closely related previously described viral species.

Parvoviruses are among the most diverse and widely distributed viruses, composed of 62 known species capable of infecting vertebrates and invertebrates, with some significant veterinary pathogens, such as porcine (Mengeling et al., 2000) and canine parvoviruses (Decaro and Buonavoglia, 2012; Parrish et al., 1988). Here, a total of 382 reads were annotated to the family *Parvoviridae*, consisting of genera *Bocaparvovirus*, *Dependoparvovirus* and *Chapparvovirus*, a proposed parvoviral genus (Yang et al., 2016). The bocaparvovirus-like reads (from Group AJ) could be further assembled into 5 contigs [rodent-associated bocaparvovirus contigs 1–5 (RBoV C1–5)] with lengths of 117–531 nt, showing 76–82% nt identity with the NS1, NP or VP1/2 genes of bocaparvovirus isolate ZJ08 (GenBank accession number: MF085373), an unclassified bocaparvovirus identified in the feces of domestic minks (Fig. 3A). A 332-nt contig (RBoV C2) annotated to the NS1 gene was used for phylogenetic analysis with other representatives of parvoviruses which showed that RBoV C2 clustered closely with PBoV-JZ08 within the genus *Bocaparvovirus*, with 82% nt identity, but was distinct from the ICTV-recognized ungulate bocaparvovirus 1 and ungulate bocaparvovirus 2 (Cotmore et al., 2014) (Fig. 3A). The chapparvovirus-like reads (from Group YQ) could be assembled into a single contig [rodent-associated chapparvovirus contig 1 (RChV C1)] with a length of 1165 nt, and phylogenetic analysis showed its closest relationship was with the NS gene of Desmodus rotundus parvovirus (DsPV) (Souza et al., 2017) with an nt identity of 72% (Fig. 3B).

Dicistroviruses are positive sense ssRNA viruses capable of infecting a wide range of arthropods (Valles et al., 2017) and also possibly rodents (Phan et al., 2011). The dicistrovirus-like reads from Group YQ were classified into aparavirus- and cripavirus-like species. The aparavirus-like reads could be assembled into 12 contigs [rodent-associated Formica exsecta virus 1 contigs 1–12 (RFexV C1–12)] with lengths of 186–2026 nt (Fig. 3C). Sequence comparisons showed the assembled contigs shared similar nt identities (87–94%) with Formica exsecta virus 1 (FexV) found in the narrow-headed ant (*Formica exsecta*) (Johansson et al., 2013), and covered almost the entire FexV genome (Fig. 3C). Comparative analysis of a 1446 nt contig (RFexV C11)
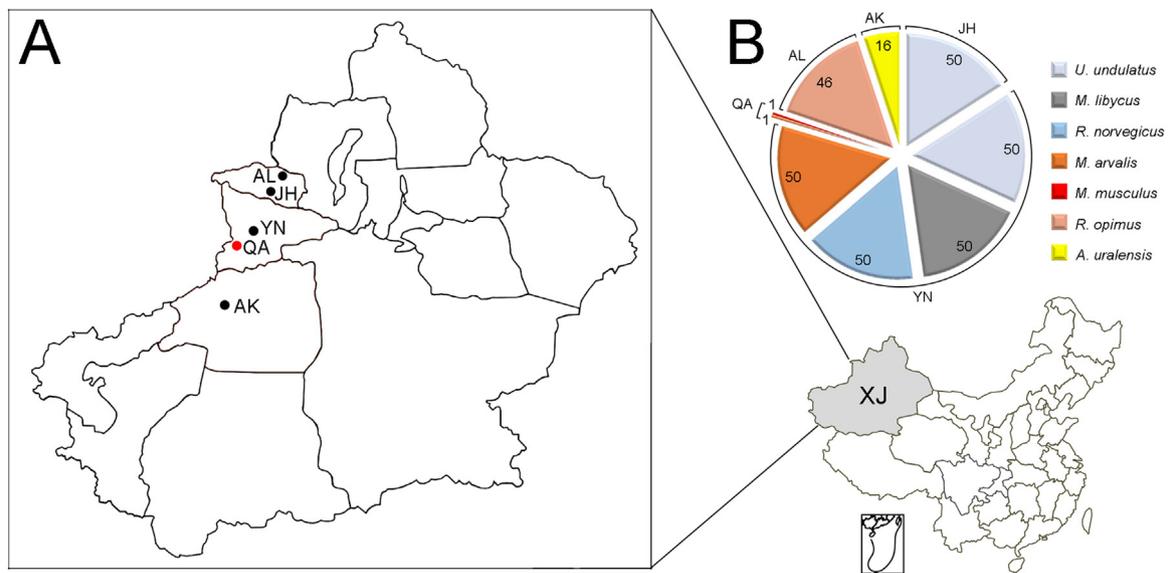
Fig. 1. Sampling information in this study. A: sampling locations; B: sample composition. XJ: Xinjiang Uyghur Autonomous Region; AL: Alashankou City; JH: Jinghe County; YN: Yining City; QA: Qapqal Xibe Autonomous County; AK: Aksu City.

corresponding to the conserved structural protein-encoding gene showed that RFexV C11 clustered closely with FexV with 94% nt identity within the genus *Aparavirus* (Fig. 3C). After assembly using the cripavirus-like reads, only one contig [rodent-associated dicistrovirus contig 1 (RDV C1)] of 1085 nt in length was obtained and showed relatedness to the non-structural polyprotein of dicistroviruses. As shown in Fig. 3D, RDV C1 clustered with a Hubei picorna-like virus 24 (HBPV), identified in myriapods previously collected in Hubei, China (Shi et al., 2016), with an nt identity of 67%, and was also closely related to bat cripavirus-P17 (BtCrV, GenBank accession number: KX644942) detected in the feces of straw-coloured fruit bats (*Eidolon helvum*) in Cameroon, as well as Wuhan arthropod virus 2 (WHAV, GenBank accession number: KX884287) previously identified in pill worms in Wuhan, China (Shi et al., 2016).

Thirty-one reads from Group YQ annotated with members of the order *Bunyavirales*, 15 of which were related to orthonairoviruses, and the remainder to peribunyaviruses. Orthonairoviruses comprise many zoonotic viruses transmitted by ticks, such as Crimean-Congo hemorrhagic fever virus (Yen et al., 1985) and Nairobi sheep disease virus (Marczinke and Nichol, 2002). The orthonairovirus-like reads were assembled into 4 contigs [rodent-associated Tamdy virus contigs 1–4 (RTAMV C1–4)], 3 of which were 158–222 nt in length and sharing 95–96% nt identity with the L segment of Tamdy virus (TAMV), a tick-borne nairovirus found in Uzbekistan (Alkhovsky et al., 2017). The remaining read was 336 nt in length and shared 96% nt identity with the S segment of TAMV (Fig. 3E). Phylogenetic analyses of the 336 nt-long RTAMV C4 revealed that RTAMV clustered closely with TAMV (Fig. 3E). The family *Peribunyaviridae* contains two genera, *Herbevirus* and *Orthobunyavirus*. the latter harbored by arthropods and able to infect mammals (Marklewitz et al., 2013). The peribunyavirus-like reads could be assembled into two contigs [rodent-associated peribunyavirus contigs 1–2 (RPeV C1–2)] with lengths of 158 and 533 nt, both of which were annotated to the M fragment of fox fecal bunyavirus (FfeV) (Adams et al., 2017; Bodewes et al., 2014) with 75% and 67% nt identities respectively (Fig. 3F). The 533 nt RPeV C2 was phylogenetically analyzed with other representatives and found to segregate with FfeV and Wuhan fly virus 1 (WhFV-1, belonging to species *Fly wubeivirus*, genus *Wubeivirus*, family *Phenuiviridae*) (Li et al., 2015a) (Fig. 3F).

Paramyxoviruses contain many members that cause lethal diseases of humans and other animals, including henipaviruses and

rubulaviruses (Amarasinghe et al., 2018; Chua et al., 2000; Nagai, 1999). Here, we identified 45 reads related to Beilong virus (BeV), a prototype of the proposed genus *Jeilongvirus* within the family *Paramyxoviridae*, found in a human kidney cell line (Li et al., 2006) but also recently detected in wild brown and black rats (Woo et al., 2016). These reads were all from Group AK, and could be further assembled into 19 contigs [rodent-associated jeilongvirus contigs 1–19 (RJV C1–19)] with lengths of 83–372 nt (Fig. 3G). By comparison with the reference sequence, these contigs could be classified into 2 groups, one contig (RJV C7, 324 nt) showing 77% nt identity with BeV, with members of the other sharing regions of 97–100% identities with BeV scattered along the complete genome of the reference strain. RJV C7, corresponding to the matrix (M) gene, was phylogenetically analyzed against reference sequences, revealing its distant relationship with other viruses within the genus *Jeilongvirus* (Fig. 3G).

2.2. Detection and complete genomic characterization of WENV

Twenty-nine reads annotated to mammarenaviruses, all from Group YQ. These could be assembled into 6 contigs with lengths of 119–398 nt, with 4 sharing 78–85% nt identities with the L gene of WENV, and 2 having 79–83% nt identity with the N gene (Fig. 4A). The 398 nt long contig was employed as a target to design nested oligonucleotide primers for PCR screening. After RT-PCR of all samples, a single brown rat from Qapqal was found positive for WENV in its lung, rectum, liver and kidney, indicative of a systemic infection. The 318 nt long amplicons from the 4 internal organs were sequenced and found to be identical. This WENV isolate was named isolate Qapqal Rattus norvegicus 1 (QARn1). Quantitative RT-PCR (qRT-PCR) showed that the virus titers in these organs were approximately the same, with about 10^8 viral cDNA copies per gram of tissue (copies/g).

The complete genome of QARn1 was successfully obtained *via* direct amplification from positive lung tissue extracts. Similar to other WENV strains, such as Rn242 in China (Li et al., 2015b) and WENV isolate Cardamones C649 in Cambodia (Blasdell et al., 2016), the complete genome of QARn1 was composed of a 7164 nt long L segment and a 3343 nt long S segment. The 2 segments had, respectively, 57 nt and 52 nt UTRs at their 5′ termini, and 33 nt and 47 nt UTRs at their 3′ termini (Fig. 4A). The 5′ and 3′ ends, constituting the promoters for polymerase entry (Buchmeier et al., 2013), exhibited high complementarity. A stable hairpin structure - a noncoding intergenic region (IGR) predicted
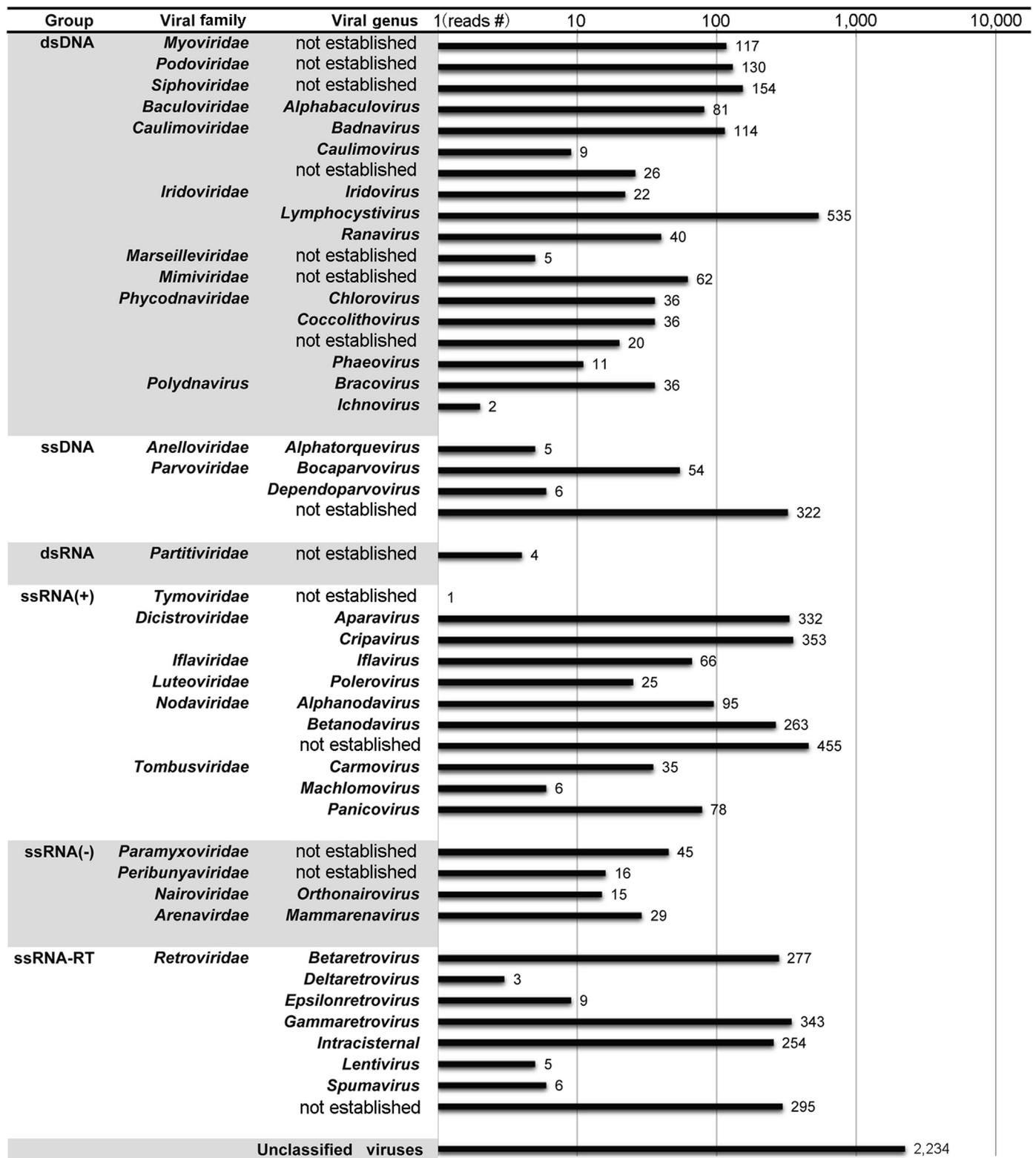
Fig. 2. Numbers of reads annotated to viruses classified by viral genus, family and nucleic acid group.

in both L and S gene segments of QARn1 (Fig. 4A), has been reported to serve as a sequence-specific signal to promote the release of the viral polymerase from the template RNA of arenaviruses (Buchmeier et al., 2013). The 109 nt IGR of the L segment was longer than that of known WENV isolates (~98 nt), and divided the complete L segment into 2 opposite-oriented open reading frames (ORF): a 276 nt long RING finger protein (Z) gene encoding a small matrix protein and a 6686 nt

long RNA-dependent RNA polymerase (RdRp) gene. In the S segment, the 62 nt IGR connected the 1478 nt glycoprotein precursor (GPC) and 1703 nt nucleoprotein (NP) genes.

2.3. Phylogenetic relationships and sequence comparison of WENV QARn1

Representatives of mammarenaviruses were aligned with the

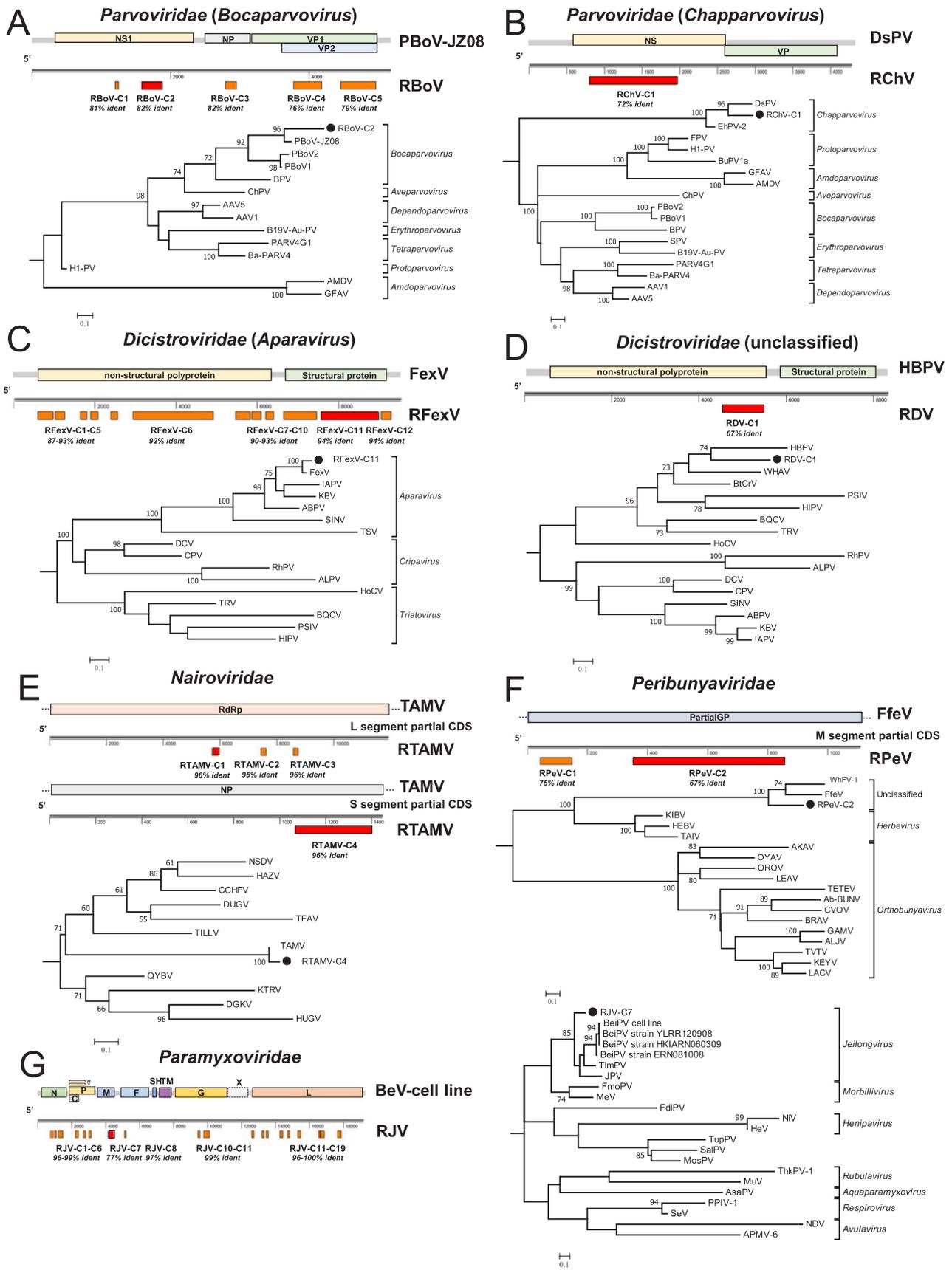**Fig. 3.** Distribution and the identities of contigs against reference sequences and their (filled circles) preliminarily phylogenetic relationship with other representatives.
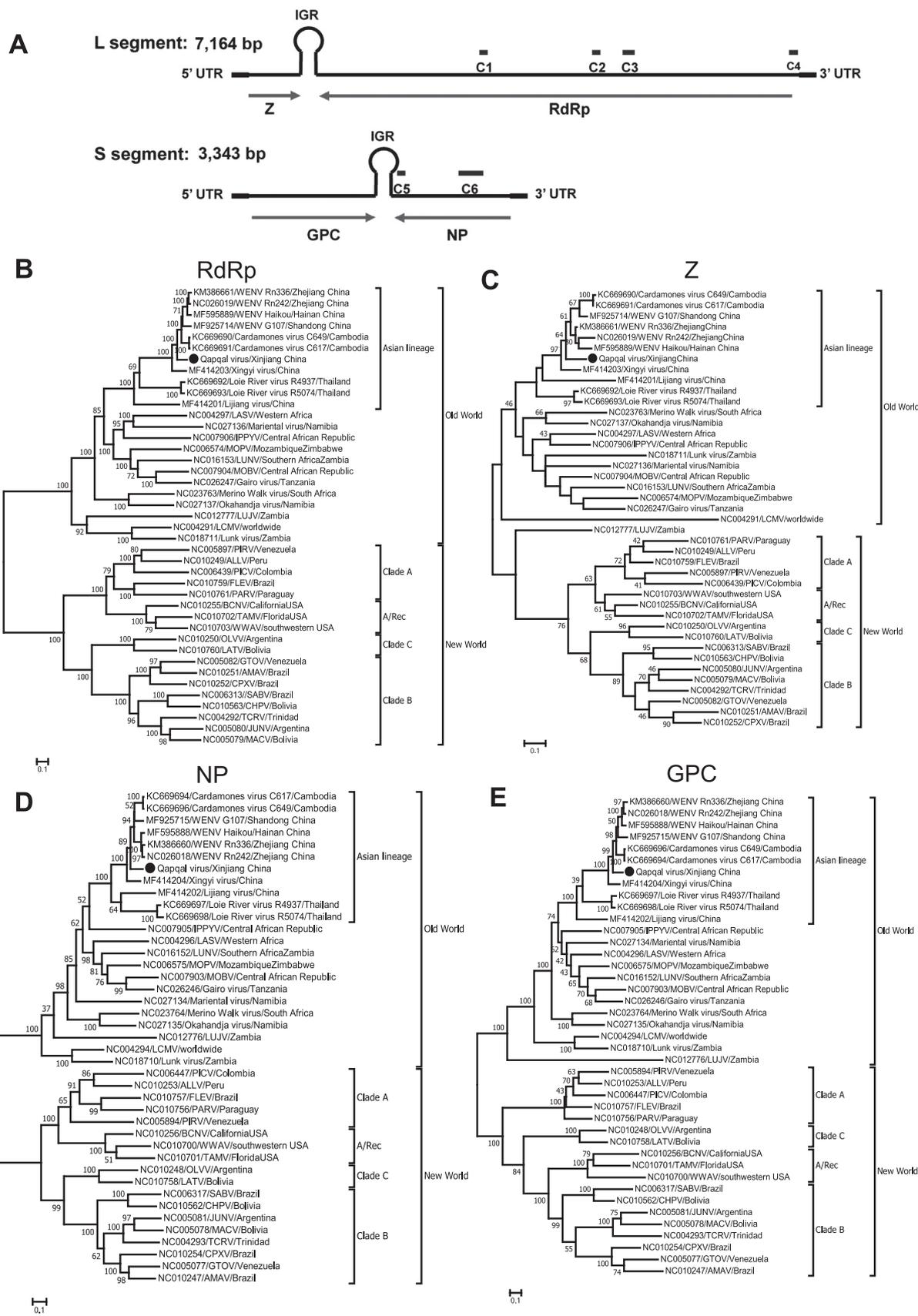
Fig. 4. Genomic structure (A) and phylogenetic relationships of WENV-QARn1 based on ORFs Z (B), RdRp (C), GPC (D) and NP (E). QARn1 is identified by a circle and bold font, and the exclusively Asian strains are indicated within gray boxes.

Table 1
Nucleotide and amino acid sequence identities (%) between QARn1 and other selected mammarenaviruses[a].

Segment/ORF	nt/aa	WENV	WENV-Cardamones	Xinyi virus	Lijiang virus	LORV	LASV	IPPYV	LUNV	MOPV	MOBV	MRLV	OKAV	LUJV	LCMV
L segment	nt	84.2	84.4	80.6	62.8	65.5	56.2	65.5	57.6	57	56.3	55.3	56.3	52.4	53.6
RdRp ORF	nt	84.4	84.2	80.3	64.1	64.8	56.9	57.1	57.2	57	57.3	53.4	55.1	51.6	51.6
	aa	89.7	89.8	86.4	65.2	66.9	53.4	55.4	54	54	54.1	49.1	51.8	45	47.5
Z ORF	nt	80.8	86.9	81.6	58.7	70.4	59.9	55.6	57.5	51.5	59.7	55	67.4	56.2	43.8
	aa	94.2	92.3	91.5	62.8	75.5	63.6	59.6	60.8	52.6	55.9	58.3	65.7	52.6	20.2
S segment	nt	86.1	86.8	83.6	70.6	71.6	66.5	68	67.6	66.3	67.1	66.4	64.1	58.7	62.3
NP ORF	nt	86.1	86.9	84.8	74.7	70	67.8	68.8	68.9	69.8	67.6	67.3	65.7	59.9	62.6
	aa	95.1	95.1	95.5	84.1	79.7	72.4	73.9	74.1	75.1	74	74.3	70.3	61	65.4
GPC ORF	nt	86.3	85.9	83.1	69.3	70.5	68.5	64.3	66.7	65.5	66.1	65.4	62.7	54	60.3
	aa	92.5	93.4	91.7	78.0	79.6	75.3	68.7	73.3	70.4	74	69.6	65.3	47.6	57.5

[a] The information of mammarenaviruses used here is shown in Table S4.

sequences obtained in this study, and maximum likelihood trees of 4 genes (RdRp, Z, GPC and NP) were generated. As shown in Fig. 4B-E, the sequences formed two groups, OW and NW, with the 4 phylogenetic trees showing similar topologies. The Asian viruses, WENV isolates Rn242, C649, LORV and two new mammarenaviruses identified in China, Xingyi virus (GenBank accession numbers MF414203-6) and Lijiang virus (GenBank accession numbers: MF414201-2), formed an Asia-exclusive cluster, tentatively named Asian lineage (Fig. 4B-E). QARn1 clustered with other WENV isolates, but had apparently diverged earlier from the remaining WENV isolates.

The genome of QARn1 was analyzed using the Pairwise Sequence Comparison (PASC) tool for calculation of sequence homology (Table S2), and identities of each gene were calculated by DNASTAR software. As shown in Table 1, QARn1 shared the highest identities (> 80.8%) with the WENV isolates at genomic and ORF levels, followed by 80.3–83.1% identities with Xingyi virus, 58.7–84.1% identities with Lijiang virus and 64.8–79.6% identities with LORV, then a distant relationship with the remaining OW viruses, including LASV and LCMV (Table 1).

2.4. Isolation of WENV QARn1 virus

Isolation of QARn1 virus was attempted both *in vitro* and *in vivo*. Sterile filtrates of homogenized lung, liver and kidney tissues of the positive rat (passage F0) were incubated with Vero-E6, BHK-21, MDCK and DH82 cells in Minimum Essential Medium (MEM) at 37 ˚C. After 5 passages, no discernible cytopathic effects (CPE) were observed in any of the cell lines, and qRT-PCR did not amplify the viral genome from the cultures. Following intraperitoneal (i.p.) injection of tissue samples, specific pathogen-free (SPF) adult female Sprague-Dawley (SD) rats (derived from *R. norvegicus* rats) showed no clinical signs over a 4-week observation period. However, virus was identified in all tissues tested (serum, lung, liver, kidney, spleen and rectum) of animals euthanized between 7 and 28 days post-infection, indicating systemic infection. Virus titers, measured by qRT-PCR, reached ~10^8 cDNA copies/ mL in sera and 10^7–10^9 cDNA copies/g in tissues at 7 d.p.i. Sera and homogenates derived from viral RNA-positive tissues (lung, liver and kidney) at 7 d.p.i. were subjected to another round of cell culture isolation but again no virus was detected by CPE or qRT-PCR after 5 serial passages. However, sera with the highest qRT-PCR titers were examined by transmission electron microscopy (TEM) and numerous spherical or pleomorphic virions with diameters of ~120 nm were observed. The particles contained a surface envelope studded with spaced spikes corresponding to glycoprotein projections (Fig. 5).

2.5. Pathogenesis and infection dynamics of WENV QARn1

To assess the pathologic effects on brown rats of QARn1, positive lung tissues of F1 animals with the highest titers (~10^9 viral cDNA copies/g) were homogenized and the supernatants were injected i.p. into another group of 12 SPF adult female rats with MEM injection of
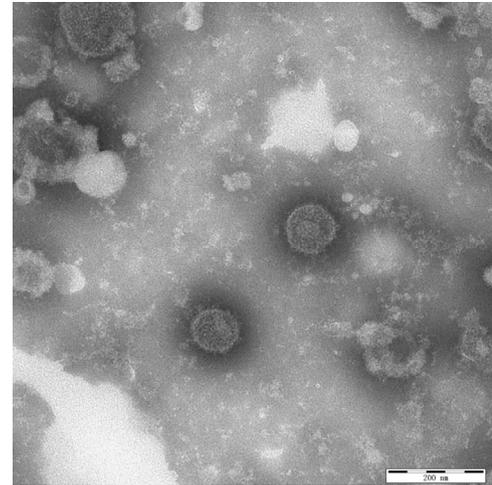

Fig. 5. Morphological observation of WENV-QARn1 virions.

another 2 as controls. All animals remained asymptomatic and were euthanized in groups of 3 over 4 weeks. Gross examination (spleen, liver, heart, kidney and rectal tissues) revealed only minor lesions in lung and thymus after 14 days with small hemorrhagic foci, with congestion apparent at 7 d.p.i., increasing over the next 2 weeks before resolving (Fig. 6). Lymphocytic and macrophage infiltration of lungs at 7 d.p.i., neutrophils in lungs at 14 d.p.i. and monocytes in lungs at 28 d.p.i. were apparent microscopically (Fig. 6), with no pathological changes being observed in spleen, ovary and intestinal tissues (cecums and rectums). Testing by qRT-PCR showed that all rats contained detectable viral RNA in at least one organ (kidney, lung, rectum, spleen, thymus, liver or brain), with virus titers peaking at 7 d.p.i. in lung and liver (~10^9 cDNA copies/g) before decreasing or disappearing.

To further investigate the dynamics of QARn1 infection, sera were taken from the tail veins of 3 rats at intervals over 4 weeks and analyzed by qRT-PCR and IgG enzyme-linked immunosorbent assay (ELISA), with confirmation by IgG Western blot (WB) analysis. As shown in Fig. 7A, viremia appeared as early as 3 d.p.i., peaking by 5–7 d.p.i. (10^5–10^7 cDNA copies/mL) before being cleared by 15 d.p.i. ELISA testing showed that IgG was first detected at 7 d.p.i. (P/N ≥ 2.1), reaching a plateau by 15 d.p.i. (P/N = ~10) (Fig. 7B). WB analysis of samples over the 28-day period showed that a QARn1 rNP band of ~66 kDa, could be detected as early as 9 d.p.i. (Fig. 7C).

2.6. Interspecies infection assessment of WENV QARn1

Three groups of SPF adult female rodent, including Kunming mice (n = 8), Syrian golden hamsters (n = 8) and albino guinea pigs (n = 4), derived from rodent species of *Mus musculus*, *Mesocricetus auratus* and *Cavia porcellus* respectively, were injected i.p. with F1 rat supernatants. A further group of suckling Kunming mice (n = 32) were injected by
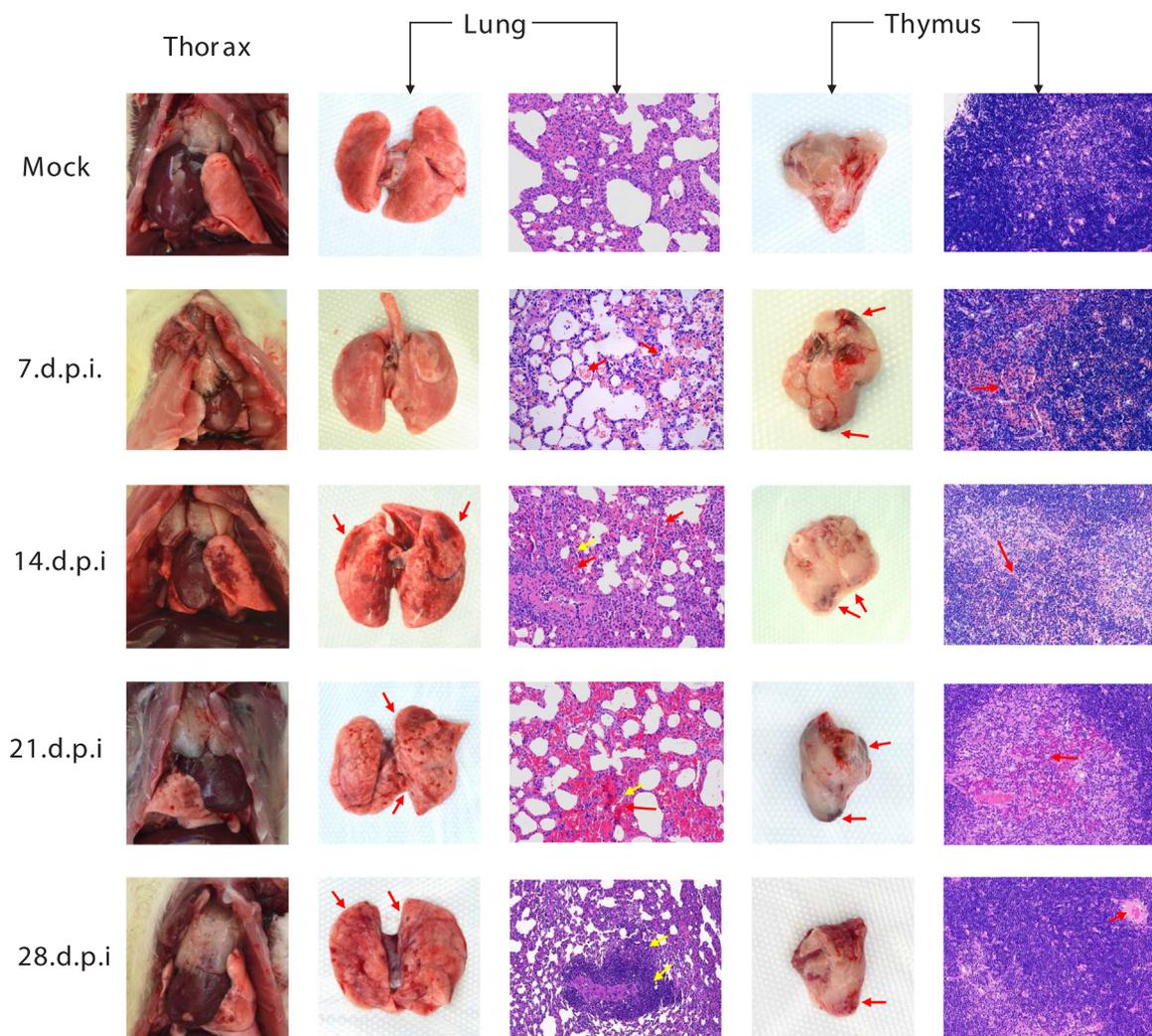
Fig. 6. Macroscopic and histological findings in WENV-QARn1-infected rats at 7 d.p.i., 14 d.p.i., 21 d.p.i. and 28 d.p.i. as well as PBS-inoculated mock control. Gross examination of thorax, lung and thymus tissues are respectively shown. In parallel, sections of lung and thymus tissues are shown with haematoxylin–eosin staining after fixation in 10% formalin, and observed at 200 × by digital sight. Red arrow: bleeding region by macroscopic and histopathological examination; yellow arrow: inflammatory cell infiltration.

the i.p. and intracranial routes. No clinical signs were observed in any during a 3-week observation period. No virus was detected by qRT-PCR in tissues from animals euthanized weekly, and only guinea pig sera at 21 d.p.i. showed a positive antibody response by WB (data not shown).

3. Discussion

As the most diverse, abundant and widest distributed mammals in the world, rodents are recognized as important natural reservoirs of many zoonotic pathogens. Their habitats frequently exist within human communities, thereby providing the opportunity for transmission of pathogens to humans and domestic animals. By 2013, more than 170 viruses had been reported in these creatures, including ones deadly to humans, such as Lassa virus and hantavirus (Luis et al., 2013). However, with viral metagenomic analysis being applied to profile the virome of rodents, the viral diversity of rodents has been dramatically expanded. Phan et al. (2011) conducted a viromic profiling of wild rodent feces in America, revealing viruses of 24 families, with circoviruses as the most abundant (Phan et al., 2011). Sachsenröder *et al.* (2014) reported a metagenomic analysis of intestinal contents from wild brown rats collected in Berlin, Germany, resulting in 34 known virus families and 75 genera, with parvoviruses and with picobirnaviruses as the most abundant species (Sachsenröder et al., 2014).

Hansen et al. (2016) sampled brown rats from Malaysia, Hong Kong and Denmark, and conducted a metagenomic investigation of feces of these animals. They found 40 eukaryote-specific virus families with virgaviruses forming the largest proportion, followed by parvoviruses (Hansen et al., 2016). The viral flora carried by house mice (Williams et al., 2018) and brown rats (Firth et al., 2014) in New York City showed highly distinct viromic compositions even though captured in a geographically limited area. Recently, a systemic viromic survey has also been conducted in rodent collected across China, which has greatly increased our knowledge of the viral community in rodents (Wu et al., 2018c). In the present study 7 species of rodents were investigated, with tissue samples as well as rectal contents being subjected to viral metagenomic analyses. Overall, the viromic analyses have identified certain viruses present in species such as parvovirus, dicistrovirus, iflavirus, and iridovirus, indicating that these viruses are common to rodents worldwide. However, some viruses have been found to vary among animal species and locations. For example, circoviruses, astroviruses and adenoviruses have been identified in previous research studies but not in this study. Instead, anelloviruses, mammarenaviruses, orthonairoviruses and peribunyaviruses were the predominant viral species identified here. In fact, neither orthonairoviruses nor peribunyaviruses have been reported previously in rodents. Both are possible arthropod viruses, since they share identities with arthropod-borne
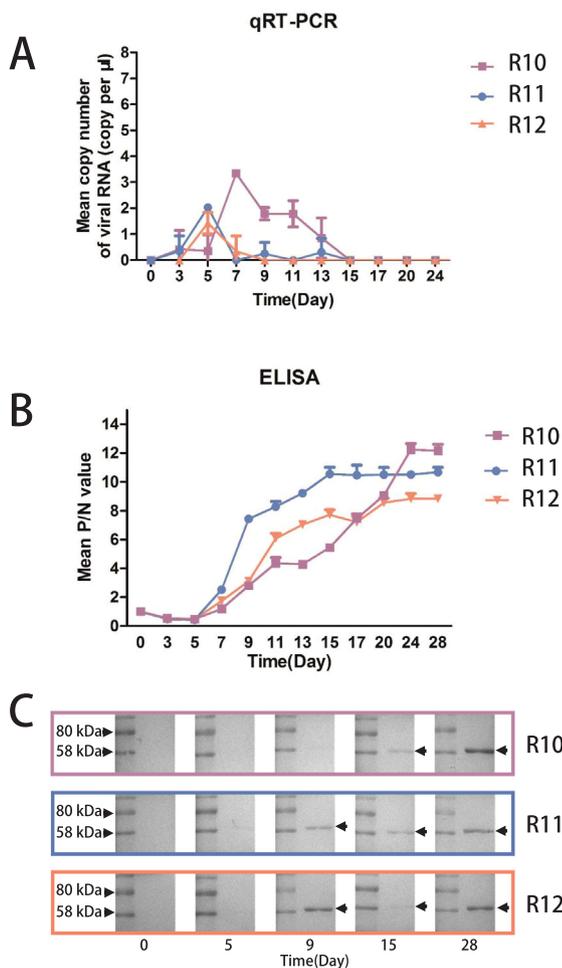
Fig. 7. IgG tests and quantification of viremia of WENV-QARn1 infected rats. SD rats were intraperitoneally inoculated with PBS (mock control) or WENV-QARn1. At the indicated time points, sera were harvested from three rat individuals (marked as R10, R11 and R12). All sera were tested by qRT-PCR (A), IgG ELISA(B) and IgG Western blot (C).

Tamdy virus and Wuhan fly virus, although the peribunyaviruses identified here also showed identity with fox fecal bunyavirus. The viral reads revealed by high-throughput sequencing-based metagenomics most likely originate from viruses that infect or are harbored by rodents, such as mammarenavirus and paramyxovirus, or organisms upon which the rodents had prayed, resulting in detection such as insect-infecting cripavirus and peribunyavirus, but the possibility cannot be excluded that some of these sequences derive from endogenized viral sequences (EVEs), either in the rodent genomes or in the genomes of other organisms, even though host genomes and free nucleic acids were removed by digestion of nuclease. Generally, results here, coupled with previous studies, have shown that rodent viromes significantly vary among host species and sampling locations, and rodent viruses are far more diverse than our previous expectations and remain largely unknown (Carroll et al., 2018; Wu et al., 2018b).

Here, we assembled the virus-like reads into contigs and conducted preliminary phylogenetic analyses of 7 mammalian or arthropod viruses. Generally, the identified aparaviruses and orthonairoviruses were variants of currently known viruses, since they showed > 87% identities to reference strains, but the bocaparvoviruses, chapparvoviruses, cripaviruses and peribunyaviruses are likely new, since they shared < 82% identities with known viruses. Of note are the paramyxoviruses, with all contigs sharing 97–100% identities with Beilong virus, a prototype of the proposed genus *Jeilongvirus* within the *Paramyxoviridae*, apart from one contig that shared only 77% identity

with the Beilong viral M gene. This latter situation might be ascribed to co-infection with two paramyxoviruses, or to a recombinant para-myxovirus, although recombination is rarely observed in para-myxoviruses (Chare et al., 2003).

Many mammarenaviruses are known to cause deadly hemorrhagic fevers in humans in Africa and the Americas, but little is known about them in Asia. The present study has focused on characterization of the newly-identified mammarenavirus QARn1. Sequence-based analyses showed that it is a new strain of WENV since, according to the species demarcation criteria to constitute a new species of *Arenaviridae* proposed by the ICTV (Radoshitzky et al., 2015), the nt identities of QARn1 were above the cut-off identities for the S (80%) and L (76%) segments separately. Additionally, QARn1 shares the same host (*R. norvegicus*) as other WENV isolates. Consistent with the high genetic diversity reported for LCMV (Albariño et al., 2010) and LASV (Bowen et al., 2000), the present study has provided evidence for the same with WENV. Among the isolates of WENV, up to 15% nucleotide divergence was observed within the L segment, and up to 13% within the S. OW mammarenaviruses show high genetic diversity in Africa, and each virus is usually associated with a single or closely related host species within a restricted area. Consequently, transmission of OW mammar-enaviruses is generally limited to endemic foci determined by the dis-tribution of the natural hosts at the species or sub-species level (Gryseels et al., 2017). LCMV, carried by the house mouse as well as hamsters, is the only globally-distributed arenavirus identified so far (Jamieson et al., 2006). According to the wide distribution and sig-nificant population of brown rats, we hypothesize that WENV is another globally-distributed arenavirus that requires further investigation else-where. To date, WENV and LORV have been sporadically reported in southern China and South-eastern Asia, and WENV could be pathogenic to humans (Blasdell et al., 2016). The identification of WENV QARn1 in this study indicates that WENV has a much wider geographic dis-tribution than this, and might also be found in Central Asia. Increasing viral surveillance and more frequent sampling of diverse wildlife has revealed that mammarenaviruses also exhibited considerable diversity in Asia, such as Xingyi and Lijiang viruses which show close relation-ship with WENV while perhaps representing a novel mammarenavirus species based on current ICTV criteria regarding genetic divergence (Table 1). Topologically, the WENVs, LORVs, Xingyi and Lijiang viruses form an Asian lineage showing a distant relationship with other OW viruses, indicating that Asia-exclusive arenaviruses likely diverged from African viruses and have independently evolved for a long time. Cur-rently the WENVs have evolved into three clades (Fig. 4B-E), corre-sponding to their distinct geographic locations more than thousands of kilometers apart, again indicating that WENVs are geographically di-vergent. Interestingly, WENVs have also shown high genetic diversity in one restricted area (Li et al., 2017), indicating further studies are needed to characterize the evolution and transmission patterns of these viruses.

Arenaviruses have been recently identified in diverse reptiles and fishes, indicating virus-host associations across the entire evolutionary history of the vertebrates (Shi et al., 2018). Although no co-divergent events have been observed among arenaviruses and their vertebrate hosts (Coulibaly-N'Golo et al., 2011; Irwin et al., 2012), there is evi-dence of host-specific adaptation of these viruses within their mam-malian or reptilian reservoirs (Zapata and Salvato, 2013). Most NW mammarenaviruses are carried by rodents within the subfamily of Sigmodontinae and Neotominae, and can be divided into four lineages: clades A, B, C and A/Rec, whereas most of the OW mammarenaviruses harbored by rodents of the subfamily Murinae form a monophyletic clade. They do, however, show clear topologic differences with the phylogenetic tree of their hosts, indicating that complex host-switching events have occurred (Fig. 4B-F) (Radoshitzky et al., 2015). Recently, mammarenaviruses clustering within the OW clade were also detected in the northern three-toed jerboa (*Dipus sagitta*) in the family of Dipo-didae, collected in North China, indicting rodents other than the

Muridae could also harbor diverse OW mammarenaviruses (Wu et al., 2018a). Within the reptarenaviruses, recombination, re-assortment and variable transmissions have been observed among different snake hosts, suggesting a more extensive mosaicism in the phylogenetic story of arenaviruses (Stenglein et al., 2015).

The Wenzhou strains of WENV have been previously isolated by cultivation in the DH82 cell line (derived from *Canis familiaris*) which was also used for QARn1 isolation. QARn1 was not rescued in this cell culture, probably because of different evolutionary adaptation of QARn1 resulting in its non-growth in this cell line. However, experimental infection *in vivo* was achieved in adult SD rats, an albino derived from wild brown rats. Mammarenaviruses generally cause persistent infections in natural hosts (some with chronic viremia) and are asymptomatic, apart from reductions in size, weight changes and mortality increases that have been found in some studies (Vitullo et al., 1987; Vitullo and Merani, 1988). Our epidemiological and pathological studies have provided definitive evidence that brown rats (*R. norvegicus*) are the natural host of WENV based on the following reasons: 1) QARn1 was detected in a wild brown rat with 10^8 viral cDNA copies/g in different organs, showing this was a natural infection of the rat; 2) QARn1 caused only mild infections in experimental animals; 3) although viremia disappeared from experimental rats after 15 d.p.i., levels of viral RNA were maintained in various organs at 28 d.p.i., indicating persistent infection with this virus *in vivo*; 4) levels of QARn1-specific IgG appeared and reached a plateau by 15–20 d.p.i. concurrent with the disappearance of viremia, showing an immune response had been mounted to clear virus from the bloodstream. Though WENVs have been found in several animal species in field studies, including *R. norvegicus*, *R. exulans*, *R. flavipectus*, *R. rattus*, *R. losea* and *N. niviventer* and one shrew species, *S. murinus* (Blasdell et al., 2016; Li et al., 2015b; Wang et al., 2017), our study showed QARn1 cannot infect the mouse, hamster or guinea pig following i.p. injection, which is suggestive of a host tropism of WENV within rodent species. Furthermore, QARn1 (or QARn1-specific IgG) was not detected by qRT-PCR and IgG WB in mice and hamsters, indicating the lack of viral replication in these species. Additionally, experiments with QARn1 did not generate productive infections in sucking mice, again suggesting that mice did not infect this virus as natural vectors. However, while QARn1-specific IgG was detected by IgG WB, virus was not detected by qRT-PCR in guinea pigs, which indicates that QARn1 can cause an adaptive immune response in the guinea pig although systemic infection did not develop. The infection dynamics of arenaviruses in their natural host has only been reported in a handful of studies, with most concluding that mammarenaviruses cause mainly acute infections but with some developing into chronic. LASV can cause chronic infection in the Natal multimammate mouse (*Mastomys natalensis*) which is the natural host of this virus (Walker et al., 1975). Morogoro virus (MORV), an African arenavirus closely related to Mopeia virus (MOPV), can cause the chronic infection in around 50% of *M. natalensis* under natural conditions (Marien et al., 2017). In laboratory experiments, infection in adult *M. natalensis* was acute: viral RNA disappeared from blood after 18 d.p.i. and from excretions after 39 d.p.i., with antibodies continuously present from 7 d.p.i. onward. Infant *M. natalensis* acquired a chronic infection with RNA and antibodies in blood for at least 3 months (Borremans et al., 2015). With regard to the NW arenaviruses, infection with JUNV could cause viral persistence in drylands lauchas (*Calomys musculinus*) (Vitullo and Merani, 1990). Catarina virus, another NW arenavirus, caused transient infection in Southern Plains woodrats (*Neotoma micropus*) while causing persistent infection in sub-adult woodrats (Milazzo and Fulhorst, 2012). While our study showed that QARn1 causes acute infections, with low viral titers, in brown rats, further investigation is required to determine whether this virus can cause chronic infection in its natural hosts.

Our results, together with previous studies, have indicated that the composition of rodent viromes varies with geographic location and host species, which contributes to the global virome project that aims to improve the capacity to detect, diagnose, and discover viruses around the world (Carroll et al., 2018). Currently, only limited studies of rodent viromes have been conducted and it remains likely that only a small fraction of the diverse viruses harbored by these animals has been uncovered. Since many viruses revealed by viral metagenomics have shown only limited identity with currently known viruses, their potential threat to humans and other animals remains unknown and therefore merits further investigation. The WENV isolates identified here provide evidence indicating widespread circulation of this virus in Central Asia. brown rats have a wide distribution worldwide and often dwell in proximity to human dwellings, thereby exposing humans to infection by excreted virus. Continuous epidemiological surveillance is critical for an understanding of any adverse effects as well as for control and prevention of transfer of rodent WENV to humans.

4. Materials and methods

4.1. Ethics statement

Sample collection and experimental infection of rodents in this study were reviewed and approved by the Administrative Committee on Animal Welfare of the Institute of Military Veterinary, Academy of Military Medical Sciences, China (Laboratory Animal Care and Use Committee Authorization, permit number: JSY-DW-2016-02). All animals were treated strictly in accordance to the Principles and Guidelines for Laboratory Animal Medicine (2006) of the Ministry of Science and Technology, China.

4.2. Sample collection and preparation

Wild rodents were collected by using glue traps at 5 locations (Fig. 1). After morphological identification by a field-trained expert, their species was then confirmed by PCR targeting the mitochondrial cytochrome *b* gene sequence (Robins et al., 2007). The rodents were collected, euthanized if not dead upon collection, and dissected immediately with their organs (kidneys, lungs, liver, rectums) being kept in dry ice before being transported to the laboratory and stored at −80 °C.

4.3. Viral metagenomic analysis

Partial specimens (~50 mg) of kidney, lung, liver and rectum with content of rodents at each sampling location, were pooled separately and by species, and subjected to viral metagenomic analysis as per our published method (He et al., 2013). Three pooled specimens were homogenized with viral transport medium (Earle's balanced salt solution, 0.2% sodium bicarbonate, 0.5% bovine serum albumin, 18 μg/L amikacin, 200 μg/L vancomycin, 160 U/L nystatin), and sequentially subjected to centrifugation, filtration for removal of cell debris and foreign materials, and digestion by DNase I (1000 U, TaKaRa) to eliminate the host genome and other free nucleic acids. Extracted viral nucleic acids were reverse transcribed and amplified by sequence-independent single primer amplification. The purified products were then subjected to Illumina sequencing in one lane at the Beijing Genome Institute (BGI, Shenzhen). All sequences generated were subjected to local BLASTn and BLASTx search against the nonredundant viral reference database of GenBank (version: 20151121). Reads with BLAST E value ≤ 10^{-5} were defined as significant and used for further analyses.

All reads relating to members of *Parvoviridae*, *Dicistroviridae*, *Paramyxoviridae*, *Peribunyaviridae*, *Nairoviridae* and *Arenaviridae* were assembled into contigs by SeqMan v7.0 and subjected to BLASTn and BLASTx search against the nonredundant viral reference database of GenBank for further analysis. All contigs were mapped against their reference sequences to indicate their corresponding genomic locations. One contig of each virus was aligned with representative viruses using ClustalW in MEGA 7.0, and preliminary phylogenetic analysis was

undertaken using the maximum-likelihood method with GTR + G + I model and evaluated with 1000 bootstrap replicates (Kumar et al., 2016).

4.4. WENV screening and complete genome sequencing

The WENV-like contigs were used to design nested RT-PCR primers targeting a 318 nt fragment of the L gene (Table S3). Total RNA of each sample was extracted manually using an RNeasy Mini kit (Qiagen), and reverse transcription was effected with the 1st cDNA synthesis kit (TaKaRa) according to the manufacturer's protocol. Amplification of cDNA used the PCR master mix (Tiangen) with the following PCR programs: 30 cycles (outer PCR) or 35 cycles (inner PCR) of denaturation at 94 °C for 30 s, annealing at 51 °C (outer PCR) or 48 °C (inner PCR) for 30 s, and extending at 72 °C for 40 s, with ddH$_2$O as a negative control. Positive PCR amplicons were ligated into pMD-18T vector (TaKaRa) and used to transfect *E. coli* DH5α competent cells (Tiangen). Six clones of each amplicons were randomly picked for Sanger sequencing on an ABI 3730 sequencer (Cometebio). To obtain full genomic sequences, overlapping primer pairs covering the terminal ends were designed based on arenavirus-like contigs and reference sequences available in GenBank (Table S4). High fidelity polymerase (NEB) was employed to amplify the target fragment and PCR amplicons were sequenced after blunt end ligation into pLB vectors (Tiangen). Contigs were then assembled into the complete genome using SeqMan v7.

To quantify RNA copies of QARn1, the Taqman quantitative RT-PCR (qRT-PCR) method was established based on a 190-nt fragment of the L segment (oligonucleotide primer sequences: Table S3). Plasmids harboring a fragment comprising 302 nt amplicons were serially diluted 10-fold and used to generate standard curves. The volume of sera and weight of tissues were measured before RNA was extracted, and total RNA was amplified and quantified using a two-step RT-PCR method with the 1st cDNA synthesis kit (TaKaRa) and Probe qPCR kit (TaKaRa) as per the manufacturer's protocols.

4.5. Genomic and phylogenetic analyses

The genomic structure of QARn1 was predicted by ORFfinder (http://www.ncbi.nlm.nih.gov/orffinder), followed by comparison with that of other WENVs. Secondary RNA structure predictions were performed with the web-based version of Mfold (http://unafold.rna.albany.edu/?q=mfold). Sequences of OW and NW mammarenavirus reference strains were retrieved from GenBank, including 33 ICTV-approved species representatives and all isolates of WENV. The complete sequences of L and S segments as well as the coding sequence of the RdRp, Z, NP and GPC genes were aligned in MegAlign, DNASTAR software, with the identity matrixes calculated separately, and further assessed using PASC (https://www.ncbi.nlm.nih.gov/sutils/pasc/viridty.cgi) with default parameters. Alignments of RdRP, Z, NP and GPC genes were generated in MEGA7 by the maximum-likelihood method based on the general time reversible model with gamma distribution and invariant sites, and evaluated with 1000 bootstrap replicates (Kumar et al., 2016).

4.6. Virus isolation and morphological observation

Lung and liver tissues of the positive rodent (passage F0) were homogenized in MEM and sterilized by passage through a 0.22 μm filter (Millipore). Filtrates were incubated with African green monkey kidney-originated Vero-E6, baby hamster Syrian kidney-originated BHK-21, Madin-Darby canine kidney-originated MDCK (all stored in laboratory), and canine kidney with malignant histiocytosis-originated DH82 cell lines (provided by Chinese Center for Disease Control and Prevention) maintained in MEM at 37 °C with daily inspections for 14 days. After 5 passages, total RNA from each passage was extracted and subjected to qRT-PCR as described above.

Eight SPF SD female adult rats were obtained from the Breeding Laboratory of Jilin University and housed separately in isolators (Tecniplast). Six rats were injected i.p. with 300 μL F0 filtrates; the remaining two received MEM as mock controls. The animals were inspected twice daily for clinical signs for up to 28 days. Rats were euthanized by anesthesia at 7, 14, 21 and 28 d.p.i. and samples of serum, brain, spleen, liver, kidney, lung and intestinal tissue were taken, homogenized, and subjected to qRT-PCR analysis. Positive tissues and sera with the highest virus titers were processed and incubated with fresh cells as described above.

Rat sera with the highest virus titers (as viral cDNA copies/g) were selected for morphological observation. Sera (~5 mL) were centrifuged at 12,000 × g for 30 min at 4 °C, and the resulting sediment was resuspended in 100 μL PBS and directly negatively stained with 5% phosphotungstic acid for observation in a Hitachi H-7650 transmission electron microscope.

4.7. Pathogenesis and infection dynamics of WENV in brown rats

Positive tissues from first-generation infected rats (passage F1) with the highest virus titers were homogenized and sterile-filtered, and 4 groups of 3 adult rats were injected i.p. with the filtrates (0.3 mL; ~10^9 cDNA copies/mL). Mock controls received MEM only. The rats were inspected daily, with single groups euthanized at 7, 14, 21 and 28 d.p.i. before dissection. Organs with significant pathological changes were photographed, then fixed in 10% formalin. Tissues were sectioned, stained with hematoxylin and eosin, and observed by light microscopy in an Eclipse Ci microscope with a digital sight DS-FI2 system (Nikon). Tail vein serum samples were taken at intervals throughout the 28 day experimental period for qRT-PCR, ELISA and WB analyses.

For ELISA, the nucleoprotein (NP; 567aa) of QARn1 was amplified and subcloned into the pET-28a (+) plasmid with a His-tag at the C terminus. The His-tagged recombinant NP (rNP) was expressed in *E. coli* BL21 (DE3) competent cells (Tiangen) by induction with 0.25 mM isopropyl-β-D-1-thiogalactpyranoside (IPTG) and purified by passage through Ni-NTA His Bind resin (Novagen). Proteins were confirmed by SDS-PAGE and WB using anti-His-tagged antibody (Santa Cruz). Corning 96-well microtitration plates were coated with purified rNP (60 ng/well), blocked with 5% skimmed milk (Promega) at 37 °C for 1 h, and incubated with 1:100-diluted test sera, in triplicate, for 1 h at 37 °C. After washing 3x with PBS-0.05% Tween, peroxidase-labeled goat anti-rat IgG (H + L) (Beyotime) was added with incubation at 37 °C for 50 min. Color development was with o-phenylenediamine (OPD) substrate (Sigma) and stopped by addition of 2 M sulfuric acid. ODs were measured at 492 nm and 630 nm in a Multimode microplate reader (Tecan) Titers were calculated from the formula OD$_{sample}$ = OD$_{492}$- OD$_{630}$.

For WB analyses, 170 ng purified rNP was boiled in 2 × protein loading buffer (TaKaRa), separated by 10% SDS-PAGE, and transferred onto nitrocellulose membranes (Millipore) which were then incubated with sera at 1:200 dilution, followed by peroxidase-labeled goat anti-rat IgG (H + L) (Beyotime). Guinea pig and hamster serum assays utilized peroxidase-labeled SPA/SPG mixture (Biodragon). The membranes were then reacted with Crescendo Western HRP Substrate (Millipore) and scanned for chemiluminescence (Tanon). For mouse serum analyses the secondary antibodies were Alexa Fluor 680 donkey anti-mouse IgG (H + L) (Life Technologies). Membranes were then scanned using the Odyssey imaging system (LI-COR).

4.8. Interspecies infection assessment

To assess the potential interspecies infection of QARn1 between different rodent species, SPF female adult Kunming mice (n = 8), Syrian golden hamsters (n = 8,) and albino guinea pigs (n = 4) (obtained from the Breeding Laboratory of Jilin University) were injected

i.p. with positive filtrates as described above. Organs were removed from animals euthanized at 7 and 14 d.p.i for assay by qRT-PCR. Serum samples taken prior to euthanasia were tested by WB as described above, but using peroxidase-labeled SPA/SPG mixture (Biodragon) for guinea pigs and hamsters, and Alexa Fluor 680 donkey anti-mouse IgG (H + L) (Life Technologies). Suckling mice (n = 32) were injected with F1 rat supernatants by the i.p and intracranial routes and, euthanized at 7 or 14 d.p.i. Organs were obtained from animals for assay by qRT-PCR.

4.9. Dataset illustration and accession numbers

Detailed information of sequences used in this study (GenBank accession numbers, abbreviations) is provided in Table S4. Sequences of all assembled contigs have been deposited in GenBank under accession numbers MG679360-MG679365 and MG685618-MG685655 (Table S1). The full genome of WENV QARn1 has been deposited in GenBank under accession numbers KY662262 for L segment and KY662263 for S segment. The raw data of Illumina sequencing have been deposited in Short Reads Archives (SRA) under accession number SRP126625.

Acknowledgements

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.virol.2019.01.010.

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