Virology 514 (2018) 9–17

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

Discovery of novel anelloviruses in small mammals expands the host range and diversity of the *Anelloviridae*

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ARTICLE INFO

Keywords:
Anellovirus
Rodent-borne virus
Bat-borne virus
Anelloviridae
Virome, and ssDNA viruses

ABSTRACT

The *Anelloviridae* comprises single-stranded DNA viruses currently grouped in sixty-eight species classified in twelve genera. They have been found in many vertebrate hosts including primates. In this study, we describe the application of the high-throughput sequencing to examine the frequency and diversity of anelloviruses in rodents, bats and opossums captured in São Paulo State, Brazil. We report a total of twenty-six anelloviruses with sixteen nearly complete genomes and ten partial genomes, which include eleven potential novel species identified in rodents (Cricetidae), bats (Molossidae and Phyllostomidae), and opossums (Didelphidae). We also propose the inclusion of two potential new genera within the *Anelloviridae* family, provisionally named *Omegatorquevirus* and *Sigmatorquevirus*, including six and three novel species of anelloviruses, respectively. In summary, this study expands the diversity and the host range of the known anelloviruses.

1. Introduction

Torque teno virus (TTV) was discovered as a possible cause of post-transfusion hepatitis in humans in 1997 in Japan (Nishizawa et al., 1997). Currently, the *Anelloviridae* family comprises about 68 species, which are non-enveloped, circular, single-stranded DNA (ssDNA) viruses with a genome of 2.1–3.9 kb in length classified into 12 genera (Biagini, 2009, 2017; Biagini et al., 2012).

Anelloviruses are associated with chronic infection without causing overt diseases and their prevalence in humans ranges from 5% to 90% (Spandole et al., 2015). Many studies have associated anelloviruses with a broad spectrum of diseases including hepatitis, multiple sclerosis, hepatocellular carcinomas, respiratory infections, blood disorders and autoimmune diseases, but these studies have not been confirmed that anelloviruses are the etiological agent of these diseases (Garcia-Alvarez et al., 2013; Mancuso et al., 2013; Masouridi-Levrat et al., 2016; Spandole et al., 2015; Tyschik et al., 2017). On the other hand, anelloviruses have been showed to be present on healthy individuals, and are commonly detected with high prevalence in several types of samples, such as blood, respiratory tract, gut, skin and human genito-urinary tract (Afkari et al., 2012; Bernardin et al., 2010; Biagini et al., 1998; Blazsek et al., 2008; Breitbart and Rohwer, 2005; Burian et al., 2011; Chan et al., 2001; Lin et al., 2000; Okamura et al., 1999; Popgeorgiev et al., 2013; Pride et al., 2012). In addition, the viral load of anelloviruses in transplant recipients has been showed as one marker of the overall state of immunosuppression (De Vlaminck et al., 2013).

Anelloviruses are spread primarily through fecal-oral transmission and saliva (Ali et al., 2002; Maggi et al., 2003; Nishiyama et al., 2014). The lack of suitable cell culture systems and animal models impairs the study of anelloviruses in laboratory settings and thus we know very

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https://doi.org/10.1016/j.virol.2017.11.001
Received 26 July 2017; Received in revised form 26 October 2017; Accepted 1 November 2017
Available online 10 November 2017
0042-6822/

little about the specific function of these viral genes and proteins in pathogenesis (Hrazdilova et al., 2016).

Currently, anelloviruses have been identified in several mammals, such as primates, dogs, cats, pigs, rodents, bats and seals (Cibulski et al., 2014; Hrazdilova et al., 2016; Ng et al., 2011a; Nishiyama et al., 2015, 2014; Okamoto, 2009; Shi et al., 2015). They have also been detected in invertebrates (i.e. mosquitoes), but probably due to blood feeding of mosquitoes obtained from vertebrate hosts (Ng et al., 2011b). Also, the Chicken anemia virus is a pathogen of chickens and has a worldwide distribution, which was included in *Anelloviridae* family (Schat, 2009).

In rodents, studies are limited to members of the subfamily Arvicolinae family Cricetidae and Muridae family (Nishiyama et al., 2015, 2014). A comprehensive characterization of the natural hosts of anelloviruses is central to understanding their host range and transmission dynamics in nature. For this reason it is necessary to perform surveillance studies in other members of the Cricetidae rodents as well as in other mammalian species. In this study, we have applied the high-throughput sequencing (HTS) approach to determine the presence of the anelloviruses in small mammals, including Sigmodontinae rodents (Cricetidae family), bats of Molossidae and Phyllostomidae families, and opossums of Didelphidae family from various geographical locations of Southeastern Brazil.

2. Materials and methods

2.1. Samples

A total of 919 specimens obtained from 13 different animal species were collected from 2008 to 2013 from rural areas of São Paulo State, South East Brazil. Individual specimens were pooled (n = 40) based on the species, sample type (i.e. tissue, blood or sera), date and place of collection (Supplementary Table 1). To visualize the geographical distribution of the pools, we used the Tableau software v.10.1.1 (Tableau, USA) with referential geographical position for map generation, and the total of host pools were represented in clusters (Fig. 1). The species of small mammals were identified using morphological characteristics keys as previously described (Bonvicino et al., 2008; Goes et al., 2016; Sikes and Gannon, 2011).

2.2. Preparation of pools

Pool samples were processed using TissueLyser II (Qiagen, USA). Each sample tube was added a 5-mm stainless steel bead (Qiagen, USA) and the samples were processed for tissue disruption during 10 min at 30 Hz using TissueLyser II (Qiagen, USA). Then, the samples were centrifuge during 5 min at 10,000 g. Subsequently, the supernatant was collected and filtered using 0.22 μm-pore-size polyvinylidene difluoride filters (Millipore, USA) to minimize the presence of eukaryotic cells and bacteria. Then, the pools were ultracentrifugeted at 180,000 × *g* for three hours at 4 °C in 30% sucrose solution (Sorvall WX90 Ultracentrifuge, Thermo, USA), and pellets resuspended in 500 μl of Hanks' balanced salt solution. To remove the naked DNA and RNA, 200 μl of the resuspended pellet from each pooled sample were digested in a cocktail with 20U of Turbo DNase (Life Technologies, USA), 25U of benzonase (Sigma-Aldrich, USA), and 0.1 mg/ML of RNase A (Life Technologies, USA) at 37 °C for 2 h in 20 μl of 10X DNase buffer (Life Technologies, USA). The viral genomes were extracted with a QIAamp viral RNA mini kit (Qiagen, USA).

2.3. Genome sequencing and assembly

The DNAs were prepared for high-throughput sequencing using RAPID module with the TruSeq Universal adapter (Illumina, USA) protocols and standard multiplex adaptors. A paired-end, 150-base-read protocol in RAPID module was used for sequencing on an Illumina HiSeq. 2500 instrument as recommended by the manufacturer. Sequencing was performed in Life Sciences Core Facility (LaCTAD) from State University of Campinas (UNICAMP), Brazil. A total of 7,059,398 to 94,508,748 paired end reads per pool were generated with 64.85–91.13% of bases ≥ Q30 with a base call accuracy of 99.9% (Supplementary Table 1). The resulting sequencing reads were cleaned and *de novo* assembled using the MetaViC pipeline (Available on https://github.com/sejmodha/MetaViC).

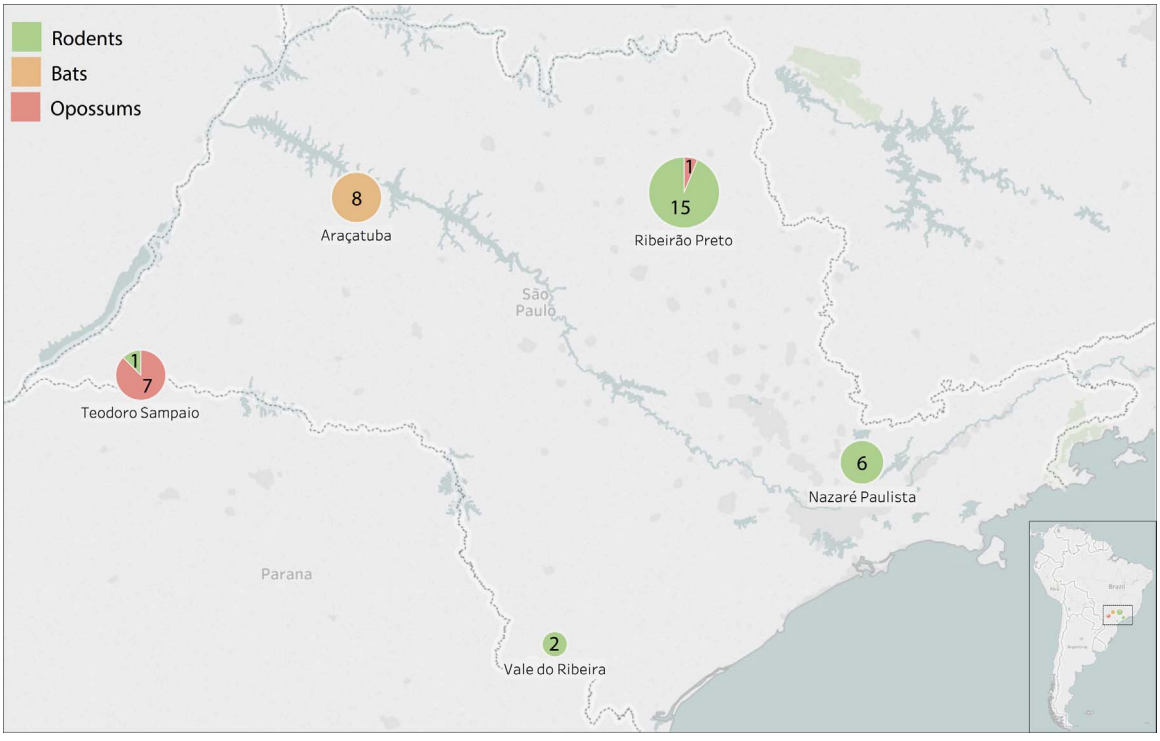

Fig. 1. Map of São Paulo State with representative graphs of the numbers of pools per host in the geographic regions of capture.

Table 1
Viruses reported in this study, including the strain name, size in nucleotides, host, ID, sample type, number of animal samples per pool (N), collection region and year. *Bold denotes prototype genome sequences.

Genus	Species/Abbreviation	Strain	ORF1	Size	Host	Host Species	ID	Sample	N	Location	Date	Genbank accession number
Deltatorquevirus	***Torque teno calomys tener virus*** **(TTCtV)**	138	Partial	1865	Rodent	*Calomys tener*	5B	Blood	38	Ribeirao Preto, SP	2008	MF541391
Sigmatorquevirus	***Torque teno carollia perspicillata virus*** **(TTCpV)**	2	Partial	1728	Bat	*Carollia perspicillata*	18	Liver	18	Araçatuba, SP	2010	MF541393
Sigmatorquevirus	***Torque teno desmodus rotundus virus*** **(TTDrV)**	182	Complete	2529	Bat	*Desmodus rotundus*	24	Liver	8	Araçatuba, SP	2010	MF541386
Sigmatorquevirus	***Torque teno didelphis albiventris virus*** **(TTDaV)**	3470	Complete	2605	Opossum	*Didelphis albiventris*	16	Serum	14	Teodoro Sampaio, SP	2009	MF541378
Omegatorquevirus	***Rodent Torque teno virus 3*** **(RoTTV-3)**	250	Complete	2538	Rodent	*Necromys lasiurus*	10B	Blood	58	Ribeirao Preto, SP	2008	MF541384
Omegatorquevirus	*Rodent Torque teno virus 4* (RoTTV-4)	188	Complete	2561	Rodent	*Calomys tener*	6B	Blood	37	Ribeirao Preto, SP	2008	MF541383
Omegatorquevirus	*Rodent Torque teno virus 4* (RoTTV-4)	117	Complete	2577	Rodent	*Mus musculus*	16B	Blood	24	Ribeirao Preto, SP	2008–2009	MF541382
Omegatorquevirus	***Rodent Torque teno virus 4*** **(RoTTV-4)**	332	Complete	2684	Rodent	*Necromys lasiurus*	10B	Blood	58	Ribeirao Preto, SP	2008	MF541376
Omegatorquevirus	*Rodent Torque teno virus 4* (RoTTV-4)	4269	Complete	2594	Rodent	*Necromys lasiurus*	11B	Blood	52	Ribeirao Preto, SP	2009	MF541379
Omegatorquevirus	*Rodent Torque teno virus 4* (RoTTV-4)	1259	Complete	2588	Rodent	*Necromys lasiurus*	9B	Blood	59	Ribeirao Preto, SP	2008	MF541380
Omegatorquevirus	*Rodent Torque teno virus 4* (RoTTV-4)	108	Complete	2577	Rodent	*Necromys lasiurus*	8B	Blood	59	Ribeirao Preto, SP	2008	MF541381
Omegatorquevirus	*Rodent Torque teno virus 4* (RoTTV-4)	319	Complete	2536	Rodent	*Necromys lasiurus*	12B	Blood	24	Ribeirao Preto, SP	2012–2013	MF541385
Omegatorquevirus	*Rodent Torque teno virus 4* (RoTTV-4)	67	Partial	1340	Rodent	*Akodon montensis*	2B	Blood	55	Ribeirao Preto, SP	2008	MF541395
Omegatorquevirus	*Rodent Torque teno virus 4* (RoTTV-4)	152	Partial	1099	Rodent	*Calomys tener*	6B	Blood	37	Ribeirao Preto, SP	2008	MF541397
Omegatorquevirus	*Rodent Torque teno virus 4 (RoTTV-4)*	2216	Partial	1047	Rodent	*Oligoryzomys nigripes*	13B	Blood	43	Ribeirao Preto, SP	2008–2009	MF541399
Omegatorquevirus	*Rodent Torque teno virus 4* (RoTTV-4)	1458	Partial	1087	Rodent	*Oligoryzomys nigripes*	35	Liver	10	Vale do Ribeira, SP	2007–2008	MF541398
Omegatorquevirus	*Rodent Torque teno virus 5* (RoTTV-5)	2	Complete	3433	Rodent	*Akodon montensis*	4B	Blood	48	Ribeirao Preto, SP	2012–2013	MF541374
Omegatorquevirus	*Rodent Torque teno virus 5* (RoTTV-5)	22	Complete	2617	Rodent	*Akodon montensis*	4B	Blood	48	Ribeirao Preto, SP	2012–2013	MF541377
Omegatorquevirus	***Rodent Torque teno virus 5*** **(RoTTV-5)**	2192	Complete	2689	Rodent	*Oligoryzomys nigripes*	35	Liver	10	Vale do Ribeira, SP	2007–2008	MF541375
Omegatorquevirus	*Rodent Torque teno virus 5* (RoTTV-5)	49	Partial	1830	Rodent	*Akodon cursor*	33	Lung	4	Nazare Paulista, SP	2009	MF541392
Omegatorquevirus	*Rodent Torque teno virus 5* (RoTTV-5)	1012	Partial	1111	Rodent	*Akodon cursor*	31	Serum	8	Nazaré Paulista, SP	2009	MF541396
Omegatorquevirus	*Rodent Torque teno virus 5* (RoTTV-5)	1594	Partial	1406	Rodent	*Necromys lasiurus*	11B	Blood	52	Ribeirao Preto, SP	2009	MF541394
Omegatorquevirus	***Rodent Torque teno virus 6*** **(RoTTV-6)**	2404	Complete	2505	Rodent	*Oligoryzomys nigripes*	13B	Blood	43	Ribeirao Preto, SP	2008–2009	MF541387
Omegatorquevirus	***Rodent Torque teno virus 7*** **(RoTTV-7)**	15	Complete	2379	Rodent	*Akodon montensis*	4B	Blood	48	Ribeirao Preto, SP	2012–2013	MF541388
Omegatorquevirus	***Rodent Torque teno virus 8*** **(RoTTV-8)**	2252	Complete	2325	Rodent	*Oligoryzomys nigripes*	35	Liver	10	Vale do Ribeira, SP	2007–2008	MF541389
Alphatorquevirus	***Opossum torque teno virus*** **(OTTV)**	13255	Partial	2220	Opossum	*Didelphis albiventris*	29	Serum	17	Teodoro Sampaio, SP	2009	MF541390

2.4. Genome characterization

Viral genomes were assessed for the genome size and ORF prediction with Geneious 9.1.2 (Biomatters, New Zealand) and CLC sequence viewer 7.7.1 (Qiagen, Germany). The ORFs were predicted using BLASTX database and based on position and nucleotide sequence similarities on NCBI database. GPMiner and Poly(A) signal miner webservers were used to predict the presence of TATA box regions and polyadenylation signals, respectively (Lee et al., 2012; Liu et al., 2003). GC-Rich regions and the molecular weight of putative proteins were predicted using Snapgene software (GSL biotech, USA) and ProtParam database, respectively (Artimo et al., 2012). The nucleotide sequences determined in this study were deposited in GenBank under the accession numbers as described in Table 1.

2.5. Phylogenetic analysis

Maximum likehood (ML) phylogenetic trees were inferred using alignments on nucleotides level of predicted ORF1 identified in the present study with ORF1 of representative members of *Anelloviridae* family. Multiple sequence alignments (MSA) were generated using MAFFT v7.309 in RevTrans 2.0 (Wernersson and Pedersen, 2003). ML trees were inferred using IQ-TREE version 1.4.3 software using GTR + I + G4 nucleotides substitution model with 1000 ultrafast bootstraps, as determined by the best-fit model based on Bayesian Information Criterion considered 88 reversible nucleotide substitution models (Kalyaanamoorthy et al., 2017; Nguyen et al., 2015). Statistical support for individual nodes was estimated using the bootstrap value. The phylogenetic trees were visualized using the FigTree software v.1.4.2. In addition, nucleotide and amino acids identifies were performed based on same alignments described above using the UGENE version 1.24.1 (Okonechnikov et al., 2012).

2.6. Frequency and diversity

We calculated the frequency and diversity of the anelloviruses based on the host species and the sample type. The frequency was calculated based on the number of positive pooled samples containing anelloviruses divided by total number of pools for their respective host, and clustered by sample type. The diversity of anelloviruses species for each host was calculated based on the amount of different anelloviruses species identified by samples type and divided by the total anelloviruses species identified reported in this study. The data were calculated using Tableau software v.10.1.1 (Tableau, USA) and the relative proportion was normalized to 100% for visualization purposes.

3. Results

3.1. Genome characterization of novel anelloviruses

The anelloviruses assemblies represent 1–33% of viral assemblies, and 0.01–2% of total of contigs assemblies. From this result, we identified 26 anellovirus contigs that were classified into eleven novel species. The viruses were identified in samples derived from rodents, bats and opossums from São Paulo State (Fig. 1), which included sixteen nearly complete genome and ten partial genomes (Table 1). The viruses identified in our study possess a ssDNA genome of 2.3–2.7 kb in length. Most anellovirus sequences had an untranslated region (UTR) with a predicted GC-rich region (> 60% GC), a putative TATA box region and a polyadenylation tail signal (Fig. 2).

All anellovirus sequences included *ORF1*, which encodes the putative ORF1 protein of 541–580 amino acids (aa) in length. The only exception is *Torque teno calomys tener virus* (TTCtV), which displayed a putative ORF1 of 337 aa, which is homologous to strains of *Rodent Torque teno virus 1* classified into same genus, the Deltatorquevirus genus. We also identified the *ORF2* that encodes a putative protein of 103–168 aa in length in *Opossum torque teno virus* (OTTV) and *Torque Teno didelphis albiventris virus* (TTDaV) (Fig. 2). *ORF3* encodes a putative protein of 127–154 aa in length and it was identified in TTCtV, TTDaV, *Torque teno desmodus rotundus* (TTDrV) and *Torque teno carollia perspicillata* (TTCpV). In addition, we identified previously uncharacterized putative ORFs called “ORF?”, potentially encoding proteins of 109–152 aa in length in *Rodent Torque teno virus 3, 4, 6* and *7* (RoTTV), TTDrV an TTCtV (Fig. 2). “ORF?” did not display homology with others anellovirus proteins, or with proteins of other viral families.

3.2. Phylogenetic analysis reveals two new genera into Anelloviridae family

To determine the phylogenetic relationships amongst the novel anelloviruses sequences and those previously described, we inferred a phylogenetic tree that included 123 complete ORF1 nucleotide sequences (Fig. 3). All known anelloviruses were split in two major groups, the primates and non-primates anelloviruses, which could be placed into 14 monophyletic clades (bootstrap values > 70%). Twelve clades corresponded to established genera within the *Anelloviridae*, which include 68 species of anelloviruses recognized by ICTV into *Alphatorquevirus*, *Betatorquevirus*, *Deltatorquevirus*, *Epsilontorquevirus*, *Etatorquevirus*, *Gammatorquevirus*, *Gyrovirus*, *Iotatorquevirus*, *Kappatorquevirus*, *Lambdatorquevirus*, *Thetatorquevirus* and *Zetatorquevirus* genus (Biagini, 2017). Furthermore, we identified two clades that were distantly related to the aforementioned genera (Fig. 3). To determine if the sequences identified in host two clades belong to previously uncharacterized genera, we calculated the pairwise genetic identity based on nucleotide level of ORF1 of all anelloviruses sequenced in our study combined with anelloviruses available in Genbank (Supplementary Fig. 1). Using the taxonomic classification criteria of ICTV (i.e cut-off values for nucleotide sequence distance: isolates 20%, species 20–55%) (Biagini et al., 2012), we confirmed that eleven sequences constituted potential novel species of anelloviruses in four genera, and that two divergent clades did indeed belong to potential two novel genera within the *Anelloviridae*, which we proposed as provisional named of *Omegatorquevirus* and *Sigmatorquevirus* (Supplementary Fig. 1).

The genus *Omegatorquevirus* is constituted by the RoTTV 4–8 identified in our study, which is composed of four anelloviruses that were previously reported, the *Rodent Torque teno virus* strain RN and AS 1014, as well as the *Mosquito VEM Anellovirus SDRB A* and *B* (Nishiyama et al., 2014; Shi et al., 2015) (Fig. 3). On the other hand, the genus *Sigmatorquevirus* that we propose here is formed by four viruses that represent three novel species, the new species TTDaV and TTCpV, as well as TTDrV that is related with *Torque teno tadarida brasiliensis virus* (TTTbV) (Cibulski et al., 2014). Interestingly, TTDrV and TTTbV were found in different hosts despite exhibiting 91% of nucleotide identity, therefore they are considered the same viral species. In addition, we have identified two novel anelloviruses species, the TTCtV and OTTV classified into the *Deltatorquevirus* and *Alphatorquevirus* genera, respectively (Fig. 3).

3.3. Frequency and diversity of anelloviruses

Eighteen of forty-five pools were positive to anelloviruses, which includes nine of the thirteen species of small mammals species tested. We observed a high frequency of anelloviruses, including more than seven different species of small mammals with frequencies higher than 40% (Fig. 4a). The most prevalent host species positive for anelloviruses were *Necromys lasiurus* and *Mus musculus* with 100% (5/5 and 1/1) of detection in the pools, followed by *Calomys tener* with 70% (2/3), all of which were detected in blood samples (Fig. 4a). On the other hand, the greatest diversity of anelloviruses species were observed in *Oligoryzomys nigripes* and *Akodon montesis* with 36% (4/11) and 27% (3/11) of the total novel anelloviruses species, respectively. Interestingly, different anelloviruses especies were found in blood, liver and in both samples of *Oligoryzomys nigripes,* showing the greatest diversity

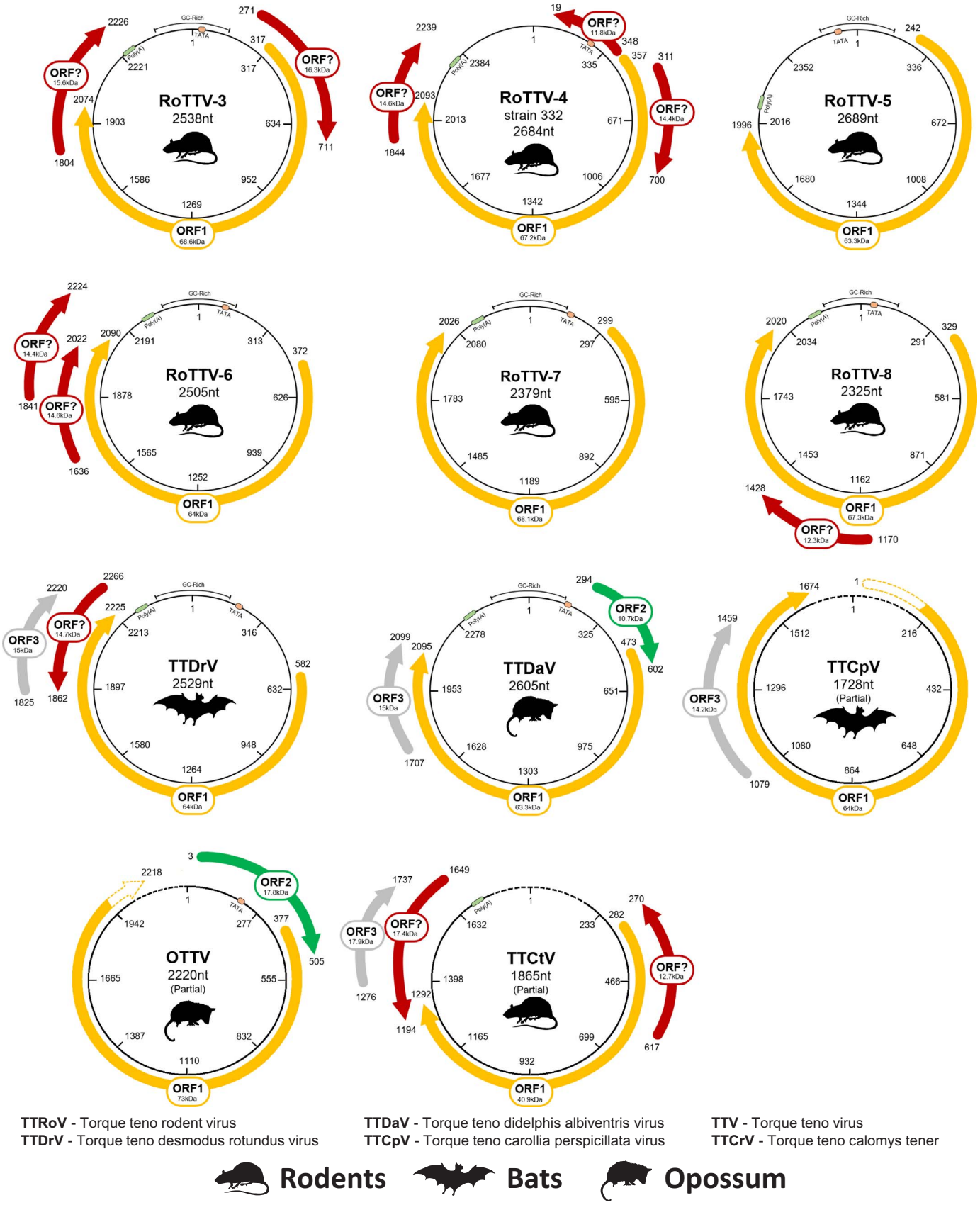

Fig. 2. Predicted genetic organization of newly discovered anelloviruses. The name and length of the determined nucleotide sequences of the viral species are shown in the center of the genomic scheme. The putative open reading frames 1–3 (ORFs) are showed in yellow, green and gray color, respectively, and the putative undetermined ORFs (ORF?) are indicated in red color. Putative TATA box region and poly A tail signal are presented with box in orange and green color. Dashed-lined indicate incomplete sequence.

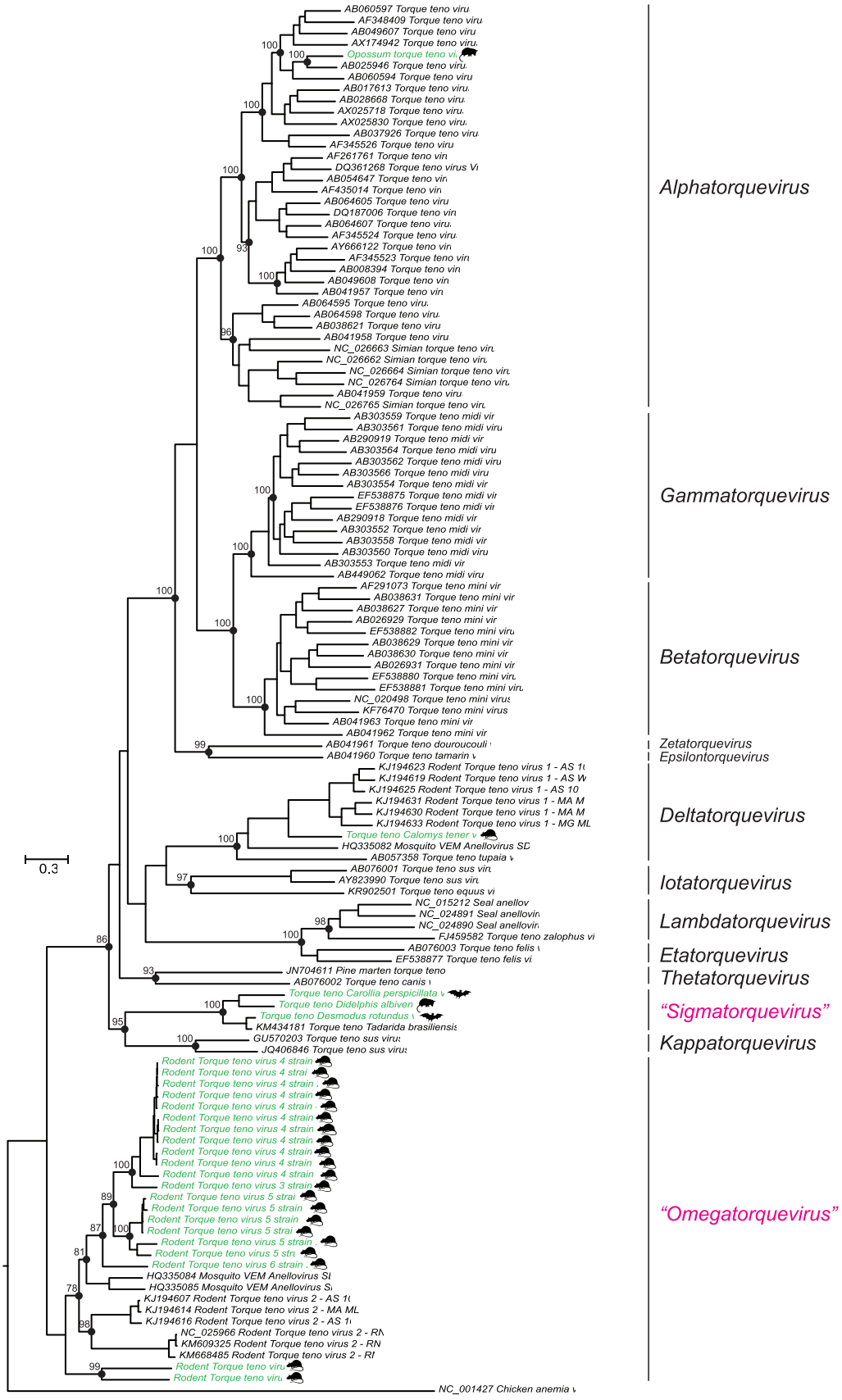

Fig. 3. Maximum likelihood phylogenies based on nucleotide level of ORF 1 showing the relationships of novel viruses identified into *Anelloviridae* family. Phylogenies are midpoint rooted for clarity of presentation. The Chicken anemia virus, an representative genome of Gyrovirus genus was used as outgroup. The scale bar indicates evolutionary distance in numbers of substitutions per nucleotide site. Branches are colored according to genus into *Anelloviridae* family. Black circle indicate the main nodes with maximum likelihood bootstrap support levels above 75% bootstrap replicates. The viruses described in this study are highlighted in green color and genus proposed highlighted in pink color.

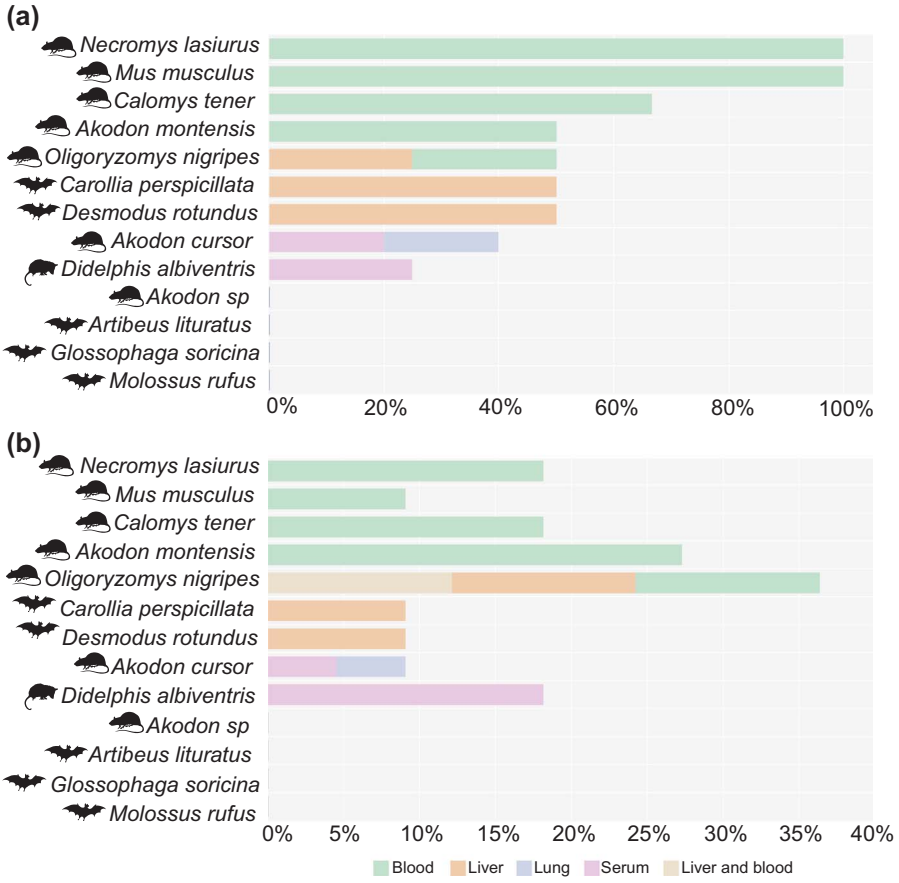

Fig. 4. Frequency and diversity of anelloviruses identified in this study. (a) Frequency of anelloviruses based on the number of positive sample pools containing anelloviruses divided by total number of pools for their respective host. (b) Diversity of anelloviruses based on the amount of different anelloviruses species identified by samples type and divided by the total anelloviruses species identified. The relative proportion was normalized to 100% for visualization purposes.

when compared to the other species (Fig. 4b).

4. Discussion

The *Anelloviridae* have been detected in blood or serum of a broad spectrum of mammals, including primates, pigs, horses, cats, dogs, rodents and bats (Cibulski et al., 2014; Hrazdilova et al., 2016; Nishiyama et al., 2014; Okamoto, 2009; Okamoto et al., 2002). Our finding, which include the discovery of eleven novel species in previously unrecognized host species and the identification of two probably new anellovirus genera highlights the diversity of this viral family.

The genome of anelloviruses can vary in length depending on the host species (Manzin et al., 2015). The size of the genomes identified in this study were consistent with previous findings, being the only exception OTTV identified in *Didelphis albiventris.* This genome was larger than expected, and it was similar in size to those of *Alphatorqueviruses* described in primates. The UTRs of the viruses were conserved similar to those of previously reported anelloviruses. This suggests that UTRs must play important regulatory roles during viral replication (Spandole et al., 2015). Features such as the TATA box and polyadenylation signal are important during transcription of viral genes (Paran et al., 2000). We were able to map the TATA box and polyadenylation signals based on ORFs position for most of the viruses identified. However, experimental work is necessary to elucidate the function of these regulators.

By combining our phylogenetic tree with pairwise distance analysis and using the classification criteria of ICTV, we assigned eleven sequences to novel anelloviruses species (Biagini, 2009, 2017; Biagini et al., 2012). Also, we identified two potential new genera within the *Anelloviridae*, which we proposed to provisional names of *Omegatorquevirus* and *Sigmatorquevirus*. These two potential genera form divergent the clades with a nucleotide distance greater than 50% to the other twelve genera. The identification of two new genera into *Anelloviridae* family increases the diversity of the described anelloviruses. Also, while the inferred phylogenetic tree indicates an origin of the anelloviruses from non-primates, there is no clear evidence to state whether anelloviruses originated as a result of co-evolution with their host during millions of years or whether anelloviruses have originated and diversified over a much shorter time scale than their hosts. Such interesting question should be answered in future studies (Okamoto et al., 2001).

Sequences belonging to the *Omegatorquevirus* genus were the most frequently identified in our study. The most common species were RoTTV-4 (eleven strains) and RoTTV-5 (six strains) detected in *Akodon montesis, Akodon cursor*, *Calomys tener*, *Necromys lasiurus*, *Mus musculus* and *Oligoryzomys nigripes*. Almost all anelloviruses were identified in the rodent subfamily *Sigmodontinae* (*Cricetidae* family), which is predominantly South American (Parada et al., 2015). Interestingly, the *Omegatorquevirus* genus form a monophyletic clade with viruses most commonly found in rodents of the *Cricetidae* family (Nishiyama et al., 2014). The exceptions are Mos VEM SDRB A and B viruses detected in mosquitoes from USA, which was suspected to be due the feed from rodents blood that potentially may be the original viral source (Ng et al., 2011b) and the RoTTV-4 strain 117 that was identified in *Mus musculus* (*Muridae* family), which have a wide distribution worldwide and anthropophilic behavior (Pocock et al., 2004). Contrasting with Rodent TTV screening study in rodents captured in United Kingdom, which showed that *Mus musculus* mouse collected were absent to the presence of anelloviruses in samples of spleen and liver (Nishiyama et al., 2014)

Bats (order *Chiroptera*) have been recognized as important sources of viruses that can potentially cause human and veterinary diseases (Brook and Dobson, 2015). However, the bat anellovirus is restricted to a single species reported in the insectivorous phyllostomide *Tadarida brasiliensis* in Southern Brazil (Cibulski et al., 2014). We have also

identified two novel anelloviruses species in *Carollia perspicillata* and *Desmodus rotundus*. These viruses form a unique monophyletic clade that we propose as *Sigmatorquevirus* genus. Another sequence belonging to the *Sigmatorquevirus* genus is TTDaV, which was derived from an opossum. Interestingly, OTTV, classified into *Alphatorquevirus* genus, was also obtained from an opossum. This is surprising because all members of *Alphatorquevirus* were identified only in primates (Spandole et al., 2015). We speculate that OTTV could be the result of anthropozoonosis, as recently proposed to Torque Teno viruses of human and swine (Ssemadaali et al., 2016). Finally, we identified in *Calomys tener* new specie within the *Deltatorquervirus* genus. This sequence clustered with other anelloviruses previously reported in rodents, shrew and mosquitos (Ng et al., 2011a; Nishiyama et al., 2014; Okamoto et al., 2001).

In sum, this is the first epidemiological study of anelloviruses in wild rodents and marsupials in the Americas, and it provides new information on the diversity and host range of anelloviruses in nature.

Acknowledgements

We thank Luiz Gustavo Betim Góes, Cristiano de Carvalho and Wagner André Pedro for the help with the capture and identification of bats in Araçatuba region, São Paulo. We thank the field team of University of São Paulo: Fábio Maués, Tatiana Ometto, Renata Hurtado and Felipe Alves Morais. We also appreciate the help in field campaigns of Márcio Schaefer, Mario Figueiredo, Felipe Morais, Jaqueline R. Silva, Paulo Paulosso and Dercílio Pavanelli. We are grateful to Edison Montilha, the Jataí Ecological Station manager for all the help, and Armando F.A. Nascimento who provide us a site for research and help on his farm Santa Gabriela in Batatais county, as well as José Teotônio (Zezinho) and the Secretary of Health from Cajuru county.

Funding

This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil (Grant number. 13/14929-1, and Scholarships No. 12/24150-9; 15/05778-5; 16/01414-1; 14/20851-8; 06/00572-0; 08/06411-4; 11/06810-9; 11/22663-6; 16/02568-2, 06/00572-0). PRM was supported by the Medical Research Council of the United Kingdom (Grant MC_UU_120/14/9).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.virol.2017.11.001.

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