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► **To cite this version:**

Edith Darcissac, Damien Donato, Benoît de Thoisy, Vincent Lacoste, Anne Lavergne. Paramyxovirus circulation in bat species from French Guiana. *Infection, Genetics and Evolution*, 2021, 90, pp.104769. 10.1016/j.meegid.2021.104769 . hal-03690904

HAL Id: hal-03690904

<https://hal.science/hal-03690904>

Submitted on 15 Jun 2022

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Research paper

Paramyxovirus circulation in bat species from French Guiana

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ARTICLE INFO

Keywords:

Paramyxoviruses

Bats

French Guiana

Phylogenetic relationships

Cross-species transmission

ABSTRACT

Bats are recognized as reservoirs of numerous viruses. Among them, paramyxoviruses, for example, Hendra and Nipah viruses, are highly pathogenic to humans. Nothing is known regarding the circulation of this viral family in bats from French Guiana. To search for the presence of paramyxoviruses in this territory, 103 bats of seven different species were sampled and screened using a molecular approach.

Four distinct paramyxovirus sequences were detected from three bat species (*Desmodus rotundus*, *Carollia perspicillata*, and *Pteronotus alitonus*) at high prevalence rates. In *D. rotundus*, two types of paramyxovirus co-circulate, with most of the bats co-infected.

The phylogenetic analysis of these sequences revealed that three of them were closely related to previously characterized sequences from *D. rotundus*, *C. perspicillata*, and *P. parnellii* from Brazil and Costa Rica. The fourth sequence, identified in *D. rotundus*, was closely related to the one detected in *P. alitonus* in French Guiana and to previously described sequences detected in *P. parnellii* in Costa Rica. All paramyxovirus sequences detected in this study are close to the *Jeilongvirus* genus.

Altogether, our results and those of previous studies indicate a wide geographical distribution of these paramyxoviruses (from Central to South America) and suggest potential cross-species transmissions of paramyxoviruses between two different bat families: Mormoopidae (*P. alitonus*) and Phyllostomidae (*D. rotundus*). In addition, their closeness to paramyxoviruses identified in rodents emphasizes the need to investigate the role of these animals as potential reservoirs or incidental hosts. Finally, the high prevalence rates of some paramyxoviruses in certain bat species, associated with the presence of large bat colonies and, in some cases, their potential proximity with humans are all parameters that can contribute to the risk of cross-species transmission between bat species and to the emergence of new paramyxoviruses in humans, a risk that deserves further investigation.

1. Introduction

Paramyxoviruses (*Paramyxoviridae* family) belong to the Mononegavirales order. They represent a large group of nonsegmented negative-strand RNA viruses subdivided into four subfamilies: *Avulavirinae*, *Metaparamyxovirinae*, *Rubulavirinae*, and *Orthoparamyxovirinae* (ICTV, 2019). Within the *Orthoparamyxovirinae*, eight genera are recognized: *Aquaparamyxovirus*, *Ferlavirus*, *Henipavirus*, *Jeilongvirus*, *Morbillivirus*, *Narmovirus*, *Respirovirus*, and *Salemvirus*. New paramyxoviruses are regularly described, constituting a fast-growing group of unclassified viruses. They have been identified from a wide range of vertebrate hosts, most of them in mammals, birds, and reptiles. In addition, the *Paramyxoviridae* family includes a vast variety of

pathogenic viruses. Common human pathogens include measles morbillivirus, mumps orthorubulavirus, and human respiroviruses (Brecht et al., 2019; Rubin et al., 2015; Henrickson, 2003; Branche and Falsey, 2016). Other human pathogenic viruses have a zoonotic origin such as the avian orthoavulavirus 1 hosted by birds, Nipah henipavirus hosted by bats of the *Pteropus* and *Rousettus* genera, as well as by cats and pigs, and Hendra henipavirus hosted by bats of the *Pteropus* genus and horses (Dimitrov et al., 2016; Middleton and Weingartl, 2012). The recent identification of Nipah and Hendra henipaviruses as causative agents of acute encephalitis in humans associated with several large outbreaks has drawn particular attention to this viral family and more specifically to their characterization and isolation in bats (Payne, 2017). Since the first identification of a paramyxovirus in a Leschenault's rousette bat

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(*Roussetus leschenaultia*) in India in 1971 (Pavri et al., 1971), and the emergence of Nipah and Hendra henipaviruses, numerous other paramyxoviruses have been detected in bats across the world (Wu et al., 2016; Yadav et al., 2016; Kurth et al., 2012; Maganga et al., 2014; Wilkinson et al., 2012; Yuan et al., 2014; Albariño et al., 2014; Amman et al., 2015; Mishra et al., 2019). Most of them, however, are not associated with human diseases. Bat paramyxoviruses infecting humans are related to the *Henipavirus* (for the Hendra and Nipah henipaviruses) and *Rubulavirus* (for the Menangle and Tioman pararubulaviruses) genera. The zoonotic potential of the other bat paramyxoviruses is still unknown.

Numerous sequences of bat paramyxovirus have yet to be assigned. However, some have been described as belonging to the *Jeilongvirus* genus whose representative members all originate from rodents (Jun et al., 1977; Mesina et al., 1974; Jack et al., 2005; Li et al., 2006; Woo et al., 2011; Woo et al., 2012; Woo et al., 2016) and that is distributed worldwide. Bat Jeilongviruses were detected in *M. mystacinus* and *P. pipistrellus* in Germany (Kurth et al., 2012), in *M. emarginatus* in Luxembourg (Pauly et al., 2017), and in various bat species in Africa as, for example, in Gabon, Zimbabwe, Rwanda and Kenya (Maganga et al., 2014; Bourgarel et al., 2018; Markotter et al., 2019; Mortlock et al., 2015; Waruhiu et al., 2017). In the same way, Asian bat paramyxoviruses Ms-ParaV/Anhui2011, B16-40, and BtMl-ParaV/QH2013 were classified in a proposed genus, *Shaanvirus*, closely related to the *Jeilongvirus* genus (Wu et al., 2016; Noh et al., 2018). The recent revision of the *Paramyxoviridae* family by the ICTV reclassified B16-40 as *Miniopron jeilongvirus* (ICTV, 2019).

In South America, paramyxoviruses related to the *Rubulavirus*, *Morbillivirus*, *Morbillivirus-related*, *Henipavirus* and *Jeilongvirus* genera have been identified in several bat species (Drexler et al., 2012; Wells, 2016). In French Guiana, located in the northern part of the Amazonian region, bats have been shown to harbor many viral strains of different viral families (*Nairoviridae*, *Circoviridae*, *Retroviridae*, *Herpesviridae*, *Papillomaviridae*, *Polyomaviridae*) (Salmier et al., 2017; Fagrouch et al., 2012; James et al., 2020), including some highly pathogenic to humans such as rabies virus (de Thoisy et al., 2016; Lavergne et al., 2016) and dengue virus (de Thoisy et al., 2009). However, nothing is known regarding the circulation of paramyxoviruses. In this context, to ascertain their presence and describe their phylogenetic relationships, we carried out molecular screening of samples of common bats collected from different places in French Guiana.

2. Materials and methods

2.1. Ethical and legal statements

All animals were captured, handled, and sampled following ASM guidelines (Sikes and Gannon, 2011) under the supervision of researchers with a French level 1 animal experimentation degree. Bats are not protected by law in French Guiana, except in protected areas. However, the experimental procedure was approved by the *Conseil Scientifique Régional du Patrimoine Naturel de la Guyane* on 10 May 2011 and 13 April 2013 and by the *Conseil National de la Protection de la Nature* on 4 April 2014. Captures that were made within protected areas (nature reserves) received approval by the *Conseil Scientifique Régional du Patrimoine Naturel de la Guyane* on 26 January 2010 and ad hoc authorizations (no. 2011–35 dated 30 May 2011, no. 35 obtained 21 March 2013, and no. 59 obtained 17 April 2013) were delivered by the Prefecture of French Guiana. The use of the genetic resources was registered with the United Nations / Access and Benefit-Sharing Clearing-House under reference ABSCH-IRCC-FR-246973-1, in compliance with the Access and Benefit-Sharing procedure implemented by the *Loi pour la Reconquête de la Biodiversité*.

2.2. Sampling areas and sample collection

Bats were captured in 14 sampling sites, of which 10 corresponded to a forest environment and four to an urban area over a 16-year period (from 2001 to 2016) (Table 1). Bats were captured at night using Japanese mist nets. Some of them were sampled for blood, saliva swabs, and/or urine before release. Others were euthanized and organs were collected (i.e., kidneys, lungs, and spleen) and stored at -80°C until needed for molecular analyses. All bats were apparently healthy. They were sexed and identified on the basis of external morphology. When necessary, species identification was confirmed via molecular techniques (by sequencing the mitochondrial *Cytochrome oxidase 1* (Borisenko et al., 2008) and/or *Cytochrome b* genes).

A total of 103 wild bats (92 euthanized and 11 released) belonging to three families and seven species (Phyllostomidae: *D. rotundus* ($n = 47$), *C. perspicillata* ($n = 33$), *A. planirostris* ($n = 8$), *S. new-species-3* ($n = 8$); Mormoopidae: *P. rubiginosus* ($n = 1$), *P. alitonus* ($n = 1$); and Molossidae: *M. molossus* ($n = 5$)) were screened for Paramyxoviruses (Table 1) with an overall proportion of 32.1% females, 62.1% males, and 5.8% individuals whose sex was not determined (Table 1). A total of 90 kidney samples, 16 lung samples, 14 blood samples, eight saliva swabs, eight spleen samples, and seven urine samples were collected (Tables 2 and 3).

2.3. RNA extraction and cDNA synthesis

For all samples, total RNA was extracted using the NucliSENS® easyMAG® automated system (bioMérieux, Marcy l'Etoile, France). cDNA was synthesized using Superscript®III reverse transcriptase (Invitrogen) and random hexamers following the manufacturer's instructions.

2.4. Paramyxovirus detection and sequences

The presence of paramyxoviruses was tested by semi-nested or nested PCRs targeting the *Polymerase* gene using different combinations of degenerate consensus primers as previously described (Tong et al., 2008). PAR primers were used to amplify viruses belonging to the *Paramyxoviridae* family; RES-MOR-HEN primers to amplify viruses of the *Respirovirus*, *Morbillivirus*, and *Henipavirus* genera; AVU-RUB primers to amplify *Avulavirus* and *Rubulavirus* viruses; and PNE primers to detect viruses of the *Pneumoviridae* family. PCR products of the expected size were cloned using the pCR™4-TOPO® TA CLONING® kit (Invitrogen) and sent for sequencing to Genewiz®, Takeley, UK (<https://www.genewiz.com/>). The sequences obtained were confirmed by similarity analysis using the NCBI BLASTn search (<http://www.ncbi.nlm.nih.gov/BLAST>). Consensus sequences were generated from three independent clones sequenced on both strands.

To join the different fragments and generate a longer contig of each identified viral sequence, different combinations of species-specific primers were derived from the sequences obtained using Primer3 (v.0.4.0) (<http://bioinfo.ut.ee/primer3-0.4.0/>) and used in PCR and/or nested PCR amplifications (Table 1S). Overlapping amplicons were generated, cloned, and sequenced as described earlier. Contig sequences were then assembled using MEGA 5.2 software (Tamura et al., 2011).

In addition, all the samples were then retested using different combinations of primers specific to each of the viral sequences obtained (Table 1S).

All newly identified paramyxovirus sequences were submitted to GenBank and the accession numbers (MW206387-95) are listed in supplementary Table 2S.

2.5. Phylogenetic analysis

All analyses were run with MEGA 5.2 (Tamura et al., 2011). The newly identified paramyxovirus *polymerase* sequences were aligned with a representative set of reference sequences. Alignments were checked

Table 1
Characteristics of the sampling sites and number of bats collected.

Bat species	Sampling sites	Environment	Years [range]	Female	Male	Undetermined	Total
<i>D. rotundus</i>	Saut Athanase	Forest	[2010–2013]	9	24	0	33
	Piste Inéri	Forest	[2013]	1	0	0	1
	Piste Bélizon	Forest	[2014]	0	1	0	1
	Cave A	Forest	[2015–2016]	1	4	0	5
	Cave B	Forest	[2015–2016]	5	2	0	7
<i>C. perspicillata</i>	Relais des papillons	Forest	[2010]	1	1	0	2
	Paracou	Forest	[2010]	0	1	0	1
	PROGT	Forest	[2015–2016]	2	1	0	3
	Counami, CIRAD	Forest	[2015]	0	2	0	2
	Cayenne	Urban	[2001–2016]	8	13	3	24
<i>A. planirostris</i>	Cave A	Forest	[2016]	1	0	0	1
	Cayenne	Urban	[2009]	0	1	1	2
<i>S. new-species-3</i>	Rémire-Montjoly	Urban	[2009]	3	3	0	6
	Camp du tigre	Urban	[2005–2008]	1	4	1	6
<i>P. alitonus</i>	Saut Athanase	Forest	[2013]	0	1	0	1
	Macouria	Urban	[2009]	0	1	0	1
	Réserve Trésor	Forest	[2010]	0	0	1	1
<i>P. rubiginosus</i>	Cave B	Forest	[2016]	0	1	0	1
<i>M. molossus</i>	Saut Athanase	Forest	[2011]	1	4	0	5
Total				33	64	6	103

Table 2
Paramyxovirus molecular screening based on kidney samples of the different bat species.

	<i>D. rotundus</i>	<i>C. perspicillata</i>	<i>A. planirostris</i>	<i>S. new-species-3</i>	<i>P. alitonus</i> or <i>P. rubiginosus</i>	<i>M. molossus</i>
PAR ^a	15/34 ^b	2/33	0/8	0/8	1/2	0/5
RES-MOR-HEN ^a	28/34	2/33	0/8	0/8	1/2	0/5
AVU-RUB ^a	0/34	0/10	0/8	0/8	0/2	0/5
PNE ^a	0/34	0/10	0/8	0/8	0/2	0/5
Bat PV ^c	29/34	6/33	ND	ND	1/2	ND

ND: not done.

^a Consensus-degenerate primers as described by Tong et al. (2008).

^b Number of positive animals / number of animals tested.

^c Paramyxovirus-specific primers of each bat species.

Table 3
Paramyxovirus molecular screening of the different types of *D. rotundus* samples.

	Urine	Spleen	Lung	Blood pellet	Saliva
PAR ^a	1/7 ^b	0/8	0/16	0/14	0/8
RES-MOR-HEN ^a	3/7	0/8	0/16	0/14	0/8
AVU-RUB ^a	0/7	0/8	0/16	0/14	0/8
PNE ^a	0/7	0/8	0/16	0/14	0/8
Bat PV ^c	5/7	0/8	0/16	0/11	0/8

^a Consensus-degenerate primers as described by Tong et al. (2008).

^b Number of positive animals / number of animals tested.

^c Paramyxovirus-specific primers of *D. rotundus*.

manually. All divergent regions including insertions and deletions were eliminated prior to analysis, and pairwise sequence identities (at the nucleotide and amino acid levels) of the partial *polymerase* coding sequences were calculated. Phylogenetic analyses were conducted using the maximum likelihood method with 1000 bootstrap replicates. A first phylogenetic reconstruction was generated using an alignment of 481 amino acids (aa) of bat paramyxovirus sequences with selected members of the *Paramyxoviridae* family (Fig. 1). The best-fitted model selected was the rtREV + G. In addition, to compare our sequences with a larger number of existing bat paramyxovirus sequences, a second phylogenetic reconstruction was generated using a smaller 143-aa fragment corresponding to the RES-MOR-HEN PCR products (Fig. 2). The model used was rtREV + G.

3. Results

3.1. Screening of paramyxovirus sequences in bat samples

To look for the presence of paramyxovirus sequences in our collection of bats, we attempted to amplify distinct regions of the RNA-dependent RNA polymerase (L) coding sequence from all the kidney samples collected ($n = 90$) using different pairs of primers, as previously described (Tong et al., 2008). Amplification products were obtained using PAR and RES-MOR-HEN primers, but none using AVU-RUB and PNE primers (Table 2). All sequences obtained were analyzed using BLAST and among them, paramyxovirus sequences were identified. For *D. rotundus*, paramyxovirus sequences were detected in 82.3% ($n = 28/34$) and 44.1% ($n = 15/34$) of the kidney samples using RES-MOR-HEN and PAR primers, respectively. In addition, one *P. alitonus* and two *C. perspicillata* individuals scored positive using the same primer couples. No amplification product was obtained from the three other bat species (*A. planirostris*, *S. new-species-3*, and *M. molossus*) (Table 2). BLAST analysis demonstrated the presence of two types of sequences in *D. rotundus* that we provisionally named “type A” and “type B”. Based on the sequences obtained, new sets of specific primers were designed to rescreen the samples (Table 1S). This allowed us to obtain paramyxovirus amplification products for 85.3% ($n = 29/34$) of the *D. rotundus* samples and 18.2% ($n = 6/33$) of the *C. perspicillata* samples as well as for one of the two *Pteronotus* spp. samples (Table 2). For *D. rotundus*, the screening was extended to the other types of available samples (urine, spleen, lung, blood, and saliva) (Table 3). Paramyxovirus sequences were only detected from the kidney and urine samples but not from any of the spleen ($n = 8$), lung ($n = 16$), blood ($n = 14$), or saliva ($n = 8$) samples (Tables 2 and 3). In the urine samples,

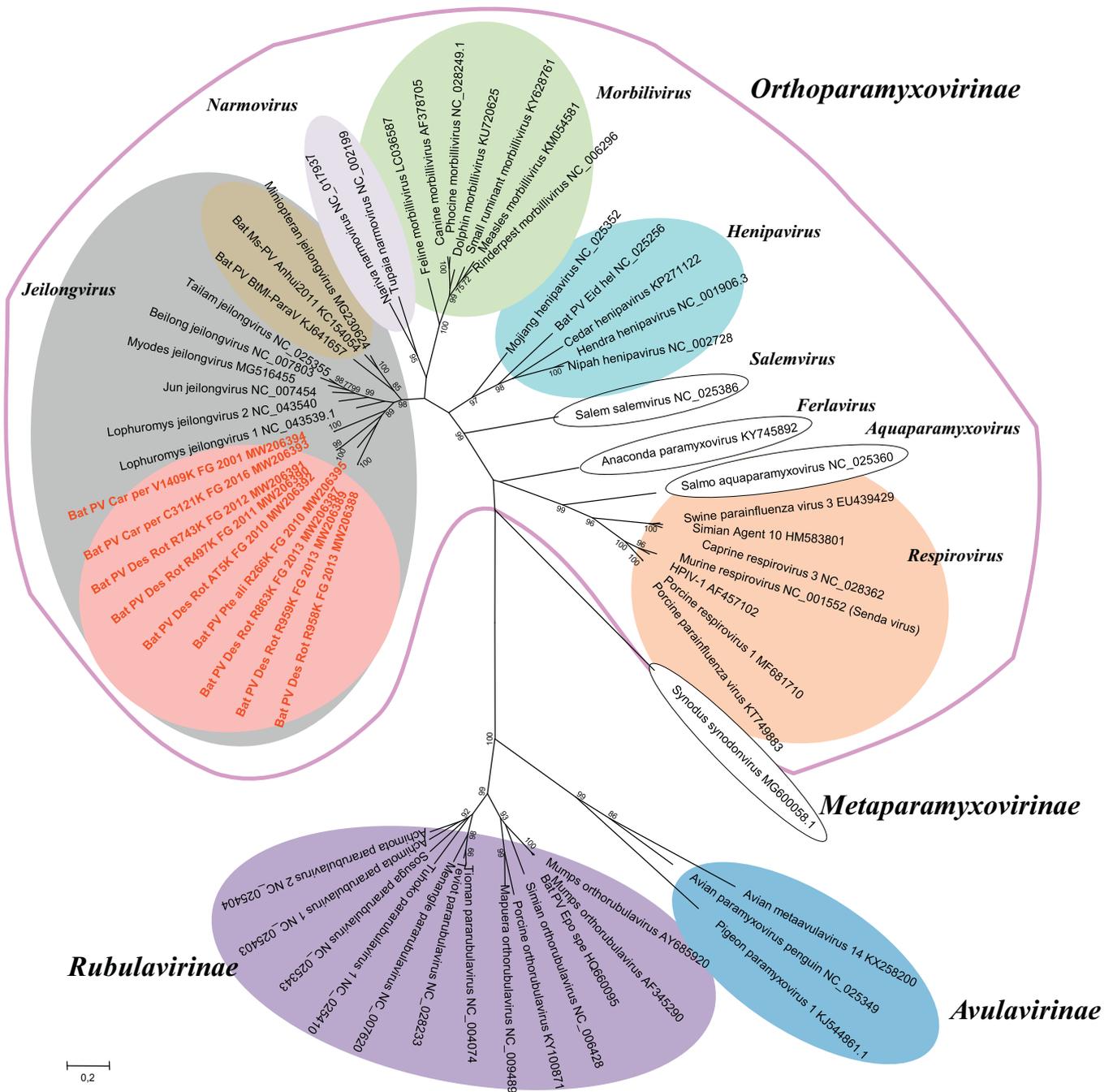


Fig. 1. Phylogenetic analysis based on 481-amino acid sequences of the RNA-dependent RNA polymerase of paramyxoviruses. The phylogenetic tree includes the nine sequences generated in this study (in red and bold) as well as 50 previously described sequences of selected members of the *Paramyxoviridae* family representative of the different subfamilies and genera. GenBank accession numbers are indicated after the viral names. The phylogenetic tree was generated using the maximum likelihood method with 1000 bootstrap replicates and the rtREV + G model. Bootstrap values above 70% are shown next to the branches. The scale bar represents the number of amino acid substitutions per site. Car per, *Carollia perspicillata*; Des rot, *Desmodus rotundus*; Epo spe, *Epomophorus species*; Pte ali, *Pteronotus alitonus*; PV, Paramyxovirus.

paramyxovirus sequences were obtained from 14.3% (1/7) of the samples using the PAR primers, 42.8% (3/7) using the RES-MOR-HEN primers, and 71.4% (5/7) using the species-specific primers (Table 3). No cross-amplification was observed when using the different primer sets specific to another bat species. Moreover, the use of specific primers made it possible to detect a greater number of positive individuals not only for *D. rotundus* and *C. perspicillata* but especially for B-type sequences, increasing the number of positive urine and kidney samples from eight to 25.

3.2. Co-infection of *D. rotundus* individuals with two distinct types of paramyxovirus lineages

As mentioned earlier, the molecular screening of *D. rotundus* kidney samples demonstrated a high prevalence of paramyxovirus (85.3%, $n = 29/37$) (Tables 2 and 4), with two lineages identified. The use of type-specific primers enabled the detection of the type A sequence in 79.4% ($n = 27/34$) of samples and the type B sequence in 73.5% ($n = 25/34$). Most of them (67.6%, $n = 23/34$) were thus co-infected with both lineages (Table 4). Similarly, the overall prevalence was 71.4% (n

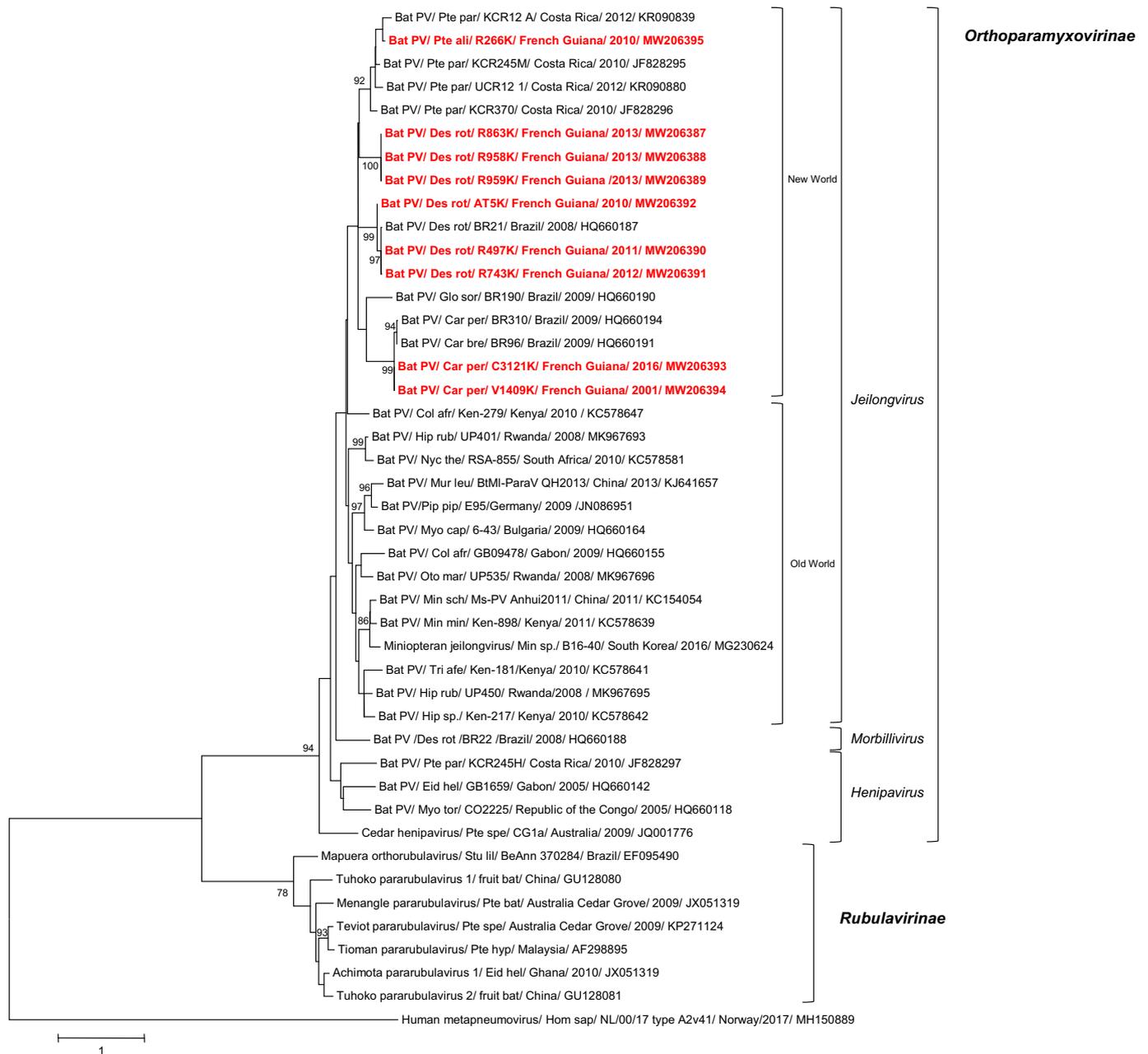


Fig. 2. Phylogenetic analysis based on 143-amino acid sequences of the RNA-dependent RNA polymerase of paramyxoviruses. The phylogenetic tree includes the nine sequences generated in this study (in red and bold) and 35 previously described bat paramyxovirus sequences. GenBank accession numbers are indicated after the viral names. The phylogenetic tree was generated using the maximum likelihood method with 1000 bootstrap replicates and the rtREV + G model. The tree was rooted with the human metapneumovirus sequence. Bootstrap values above 70% are shown next to the branches. The scale bar represents the number of amino acid substitutions per site.

Car bre, *Carollia brevicauda*; Car per, *Carollia perspicillata*; Col afr, *Coleura afra*; Der rot, *Desmodus rotundus*; Eid hel, *Eidolon helvum*; Glo sor, *Glossophaga soricina*; Hip rub, *Hipposideros ruber*; Hip sp., *Hipposideros sp.*; Hom sap, *Homo sapiens*; Min min, *Miniopterus minor*; Min sch, *Miniopterus schreibersii*; Min sp., *Miniopterus sp.*; Mur leu, *Murina leucogaster*; Myo cap, *Myotis capaccinii*; Myo tor, *Myonycteris torquata*; Nyc the, *Nycteris thebaica*; Oto mar, *Otomops martiensseni*; Pip pip, *Pipistrellus pipistrellus*; Pte ali, *Pteropus alitonus*; Pte bat, Pteropid bat; Pte hyp, *Pteropus hypomelanus*; Pte par, *Pteronotus parnellii*; Pte spe, Pteropus species; Stu lil, *Sturnira lilium*; Tri afe, *Triaenops afer*; PV, paramyxovirus.

= 5/7) in urine samples (Table 3 and Table 4). Type A and type B sequences were equally detected, with 57.1% of samples being positive ($n = 4/7$). In addition, 42.8% ($n = 3/7$) of urine samples were co-infected (Table 4).

3.3. Generation of longer sequences

Based on the PAR and RES-MOR-HEN sequences obtained, new combinations of primers were designed to generate overlapping

segments and obtain longer sequences (Table 1S). Using these new primer pairs, three 1537-nucleotide sequences and three others spanning 1751 nucleotides from *D. rotundus* as well as two 1733-nucleotide sequences from *C. perspicillata* and one covering 1715 nucleotides from *P. alitonus* were obtained. Informal virus names were given to the sequences (Table 2S). Novel paramyxoviruses were named according to the host order (Bat) and viral name (PV for Paramyxovirus) followed by the bat species from which the virus was identified (first three letters each of the genus and species), the identification code of the animal

Table 4
Molecular screening of type A and type B paramyxovirus sequences in *D. rotundus*.

	Kidney		Urine	
	n ^a	%	n	%
Paramyxovirus type A ^b	27/34	79.4%	4/7	57.1%
Paramyxovirus type B ^c	25/34	73.5%	4/7	57.1%
Co-infection	23/34	67.6%	3/7	42.8%
Total	29/34	85.3%	5/7	71.4%

^a Number of positive animals / number of animals tested.

^b Specific primers targeting type A paramyxovirus sequence of *D. rotundus*.

^c Specific primers targeting type B paramyxovirus sequence of *D. rotundus*.

collected, the country of collection (FG for French Guiana), and the year of sampling. As an example, Bat PV Car per V1409K FG 2001 stands for Bat paramyxovirus from *Carollia perspicillata*, animal V1409K captured in French Guiana in 2001.

3.4. Sequence identities

Pairwise sequence comparison was performed on 1449-bp/481-aa alignments of the RNA-dependent RNA polymerase coding sequences (Table 3S). All sequences obtained herein differed from each other at the nucleotide level, showing 71.2–99.7% sequence identity. However, three sequences from *D. rotundus* (type A, Table 3S) were identical at the amino acid level (100%) and very close (99.4–99.7%) at the nucleotide level. The three other sequences from *D. rotundus* (type B, Table 3S) were also very close to each other, showing from 90.7 to 99.3% nucleotide identity and from 97.7 to 99.6% amino acid identity. The two types of sequences found in *D. rotundus* (A and B) showed 71.7–72.1% nucleotide identity and 77.8% amino acid identity. Type A sequences from *D. rotundus* had 72.1–75.1% nucleotide identity and 74.8–80.9% amino acid identity with the paramyxovirus sequences identified from the other French Guiana bat species. Furthermore, as compared with other members of the *Paramyxoviridae* family, the closest sequence, presenting 72.3–72.4% and 77.3% identity at the nucleotide and amino acid levels, respectively, was the Lophuromys virus 1 sequence (accession number NC_043539) (Table 3S). The second type of sequences (type B) differed from the other sequences of French Guiana bat species with an identity level ranging from 71.0 to 73.1% in nucleotides and 75.5 to 78.2% in amino acids. As for type A sequences, the highest level of nucleotide (69.8–70.8%) and amino acid (75.9–76.5%) identity was also obtained with the Lophuromys virus 1 sequence compared with other members of the *Paramyxoviridae* family (Table 3S).

The two paramyxovirus sequences from *C. perspicillata* presented 97.9% nucleotide identity and 99.6% amino acid identity. These sequences differed from the other sequences from French Guianese bat species with an identity ranging from 71.0 to 72.9% at the nucleotide and 74.8 to 77.8% at the amino acid levels. They showed the highest level of identity, ranging from 70.9 to 71.4% in nucleotides and 76.5 to 76.9% in amino acids, with Lophuromys virus 1 compared with the other members of *Paramyxoviridae* (Table 3S).

The viral sequence of *P. alitonus* differed from those of the other bat species in French Guiana with an identity ranging from 72.6 to 75.1% in nucleotides and 77.5 to 80.9% in amino acids. It showed the highest level of identity with one of the sequences identified in *D. rotundus* (Bat-PV/ Des rot/ R863K/ French Guiana/ 2013/ type A) (Table 3S). Compared with the other members of the *Paramyxoviridae* family, the *P. alitonus* sequence showed the highest level of nucleotide identity (72.5%) with the sequence batMs-PV Anhui from China and 81.3% in amino acids with the jeilongvirus Lophuromys virus 1 sequence (Table 3S).

The second analysis carried out on smaller sequences, corresponding to the RMH fragment (431 bp and 143 aa), allowed us to include previously described New World paramyxovirus sequences and

Jeilongviruses described worldwide. The three sequences identified in *D. rotundus* (type A) showed the highest level of identity, ranging from 71.7 to 71.9% in nucleotides and 77.6% in amino acids, with the sequence of *P. parnellii* paramyxoviruses from Costa Rica (JF828295).

The type B sequences identified in *D. rotundus* (type B) showed the highest levels of identity, ranging from 89.8 to 94.0% in nucleotides and 95.8 to 99.3% in amino acids, with Bat PV/ Des rot/ BR21/ Brazil 2008 (HQ660187), a paramyxovirus from a *D. rotundus* from Brazil. The two paramyxovirus sequences from *C. perspicillata* presented the highest level of identity, ranging from 89.1% to 90% in nucleotides, with *C. perspicillata* paramyxovirus sequence (HQ660194) and *C. brevicauda* paramyxovirus sequences (HQ660191) both from Brazil and 97.2% in amino acids with *C. brevicauda* paramyxovirus sequence (HQ660191). The *P. alitonus* sequence showed the highest level of identity with a *Pteronotus* paramyxovirus sequence from Costa Rica (JF828295) (80.7% in nucleotides) and 90.9% in amino acids with two *Pteronotus* paramyxovirus sequences from Costa Rica (KR090839 and JF828295) (Table 4S).

3.5. Phylogenetic analyses

Fig. 1 presents the phylogenetic analysis based on a 481-aa fragment of the RNA-dependent RNA polymerase coding sequence common between the newly characterized bat paramyxoviruses and the representative sequences of each genus of the *Paramyxoviridae* family as well as some unclassified paramyxoviruses available in the databases. Almost all nodes of the tree are highly supported (bootstrap values >70%) and the main clades, *Rubulavirinae*, *Avulavirinae* and those within the *Orthoparmyxovirinae* subfamily, can be recognized. All new sequences detected here fall within the *Orthoparmyxovirinae* subfamily in a highly supported (bootstrap value = 89) monophyletic clade close to viruses belonging to the *Jeilongvirus* genus and the *Shaanvirus* genus proposed herein.

The second phylogenetic analysis, performed on smaller sequences of 182 amino acids of the RNA-dependent RNA polymerase, is restricted to bat paramyxoviruses and allows us to incorporate other sequences from bats, including those from the New World (Fig. 2). The two types of viral sequences identified from *D. rotundus* belong to two distinct subclades: the first is only composed of the three type A sequences and is related to four paramyxovirus sequences identified in *Pteronotus* from Costa Rica (JF828295, KR090839, JF828296, KR090880); the second comprises the three type B sequences as well as the sequence previously identified from a Brazilian *D. rotundus* (HQ660187). The two newly identified sequences from *C. perspicillata* are associated with formerly identified Phyllostomidae sequences from *C. perspicillata* (HQ660194) and *C. brevicauda* (HQ660191) as well as from *Glossophaga soricina* (HQ660190) from Brazil. Finally, the sequence identified from *P. alitonus* belongs to the subclade of Mormoopidae viruses within which previously identified sequences from *P. parnellii* (JF828295, KR090839, JF828296, KR090880) from Costa Rica are grouped (Fig. 2). All Jeilongvirus sequences from bats, including those described in this study, roughly cluster according to geographical origin within an old world and new world clades that are, however, not fully supported (bootstrap value <70).

4. Discussion

The role of bats as a natural reservoir of numerous viruses, some with a high impact on human health, has strengthened the need for surveillance programs in bat populations to investigate their role in emergence phenomena (Allen et al., 2017; Phelps et al., 2019; Johnson et al., 2020). Given that they harbor a wide variety of paramyxoviruses, exhibit continuous viral shedding, and show signs of organ-specific replication and absence of associated pathogenicity, bats were identified as natural reservoirs of paramyxoviruses (Drexler et al., 2012).

In this study, we made use of a molecular approach to investigate the

presence of paramyxoviruses in 103 bats from seven different species sampled in French Guiana. We characterized four distinct sequences from three species: *D. rotundus*, *C. perspicillata*, and *P. alitonus*. For three of them, closely related sequences have already been described from the same species or closely related ones (*D. rotundus*, *C. perspicillata* / *C. brevicauda*, and *P. parnellii* bats, respectively) from Brazil and Costa Rica (Drexler et al., 2012). The fourth sequence corresponds to a new paramyxovirus sequence from *D. rotundus* (type A), which has not previously been characterized from this species but is closely related to *P. parnellii* viral sequences (Drexler et al., 2012). These results, added to previous findings (Drexler et al., 2012), thus support the wide distribution of these viruses in New World bat populations, not only at a local scale (French Guiana) but also at a continental one.

The use of specific primers of each viral sequence identified revealed high prevalence rates in *D. rotundus* in both kidney (85.3%) and urine (71.4%) samples. In *C. perspicillata*, the prevalence rate observed was 18.2% in kidney. Using the same molecular approach, these differences in prevalence rates between species have already been reported, ranging from 0.7% (*C. perspicillata*) up to 61% (*M. francoismoutoui*) as well as between species of the same genus (Drexler et al., 2012; Dietrich et al., 2015). Indeed, Hendra virus was detected with a 1.76% prevalence in *P. alecto* in Australia but not in other pteropid bats (e.g., *P. poliocephalus*, *P. scapulatus*) (Edson et al., 2015). Prevalence rates also vary depending on the type of sample tested. While excretion of Hendra henipavirus in pteropid bats was detected in 90% of urine samples, using a qRT-PCR approach, it was detected in only 5% of oral samples (Edson et al., 2015). According to Drexler et al., paramyxoviruses are mainly detected in the spleen, liver, and kidney (Drexler et al., 2012). Here, paramyxovirus sequences were detected only in kidney and urine samples but not in the spleen, lung, blood, or saliva samples of *D. rotundus*, for which these different types of samples were tested, even for individuals with positive kidney samples. This is consistent with former results on bat viromes, for which no paramyxovirus sequence was detected in saliva and feces samples of *D. rotundus* and *M. molossus* (Salmier et al., 2017). These results suggest that, depending on the virus, the site of replication and mode of excretion may vary.

From a phylogenetic viewpoint, all the sequences identified herein belong to a unique, highly supported, monophyletic clade (Fig. 1). This clade is closely related to the *Jeilongvirus* genus that includes rodent paramyxoviruses such as Beilong, Jun, Lophuromys 1 and 2, Myodes, and Tailam jeilongvirus as members (Woo et al., 2012; Woo et al., 2016; Vanmechelen et al., 2018), and Miniapteran jeilongviruses such as Ms-ParaV/Anhui2011, BtMI-ParaV/QH2013, and B16-40 (Wu et al., 2016; Noh et al., 2018). As *Jeilongvirus* is a new genus defined by ICTV in 2019, many unassigned bat paramyxovirus sequences could belong to it, and it is very likely that more bat-derived *Jeilongvirus* remain to be characterized (Drexler et al., 2012; Berto et al., 2018). Due to the multiplicity of their hosts (including bats), paramyxoviruses are distributed worldwide (Drexler et al., 2012; Hayman, 2016). While in Central and South America, the greatest rate of viral diversity is expected (Hayman, 2016) and that there is a great richness of bat species in French Guiana (106 bats; Catzeflis, 2020), the distribution of paramyxoviruses in bat populations likely remain largely under-explored. Given that we did not obtain any amplification products from four species (*P. rubiginosus*, *A. planirostris*, *S. new-species*, and *M. molossus*), further investigations are needed to better assess their diversity, distribution, and evolutionary history in bats. Furthermore, the identification of two distinct clades with *Jeilongvirus* sequences from bat of old and new world, even if not supported, could indicate a geographic diversification process. Nevertheless, this hypothesis should be confirmed by the acquisition of longer sequences and further phylogenetic analyses.

As mentioned earlier, two types of paramyxovirus sequences were identified in *D. rotundus*. With 77.8% amino acid identity between them on the fragment studied, the two types of sequences identified from *D. rotundus* correspond to two distinct viral species. They both circulate at high rates, leading to a high level of co-infections (67.6%, based on

kidney samples). The co-circulation of distinct viruses was already observed in *M. francoismoutoui* bat populations on Reunion Island with the identification of four distinct paramyxovirus lineages (Dietrich et al., 2015). The presence of two types of paramyxovirus in *D. rotundus*, with one close to the paramyxovirus sequences identified in *Pteronotus*, implies a potential cross-species transmission. It has been suggested that bats are preferential donors of paramyxoviruses as compared with other hosts (mammals and birds) (Drexler et al., 2012). Whether it is the hematophagous bats *D. rotundus*, the frugivorous *C. perspicillata*, or the insectivorous *P. alitonus*, all harbor paramyxoviruses and live in huge multispecies colonies (Carter and Leffer, 2015; Williams, 1986; Solari, 2016; Catzeflis, 2020; Dávalos, 2006; Pavan et al., 2018). Moreover, given that some paramyxoviruses can be stable in the environment for up to a few days depending on environmental conditions (Fogarty et al., 2008), they could be highly concentrated in roosting sites. Furthermore, direct contacts during social interactions, such as feeding or bites (Carter and Leffer, 2015; Johnson et al., 2014; Williams, 1986), or indirect contacts through the excretion of viruses through urine or feces (Plowright et al., 2008) are known risks of cross-species transmissions (Thibault et al., 2017). All these factors contribute to the transmission and maintenance of viruses such as rabies in *D. rotundus* (de Thoisy et al., 2016; Johnson et al., 2014) and could promote the cross-species transmission of paramyxoviruses.

Viral emergence has been associated with the disruption of ecosystems related to the increasing and unsustainable use of natural resources and landscape modifications by humans (Thibault et al., 2017; García-Morales et al., 2016). Plowright et al. have shown that environmental disturbances induce nutritional stress in bat hosts and increase the risk of transmission of paramyxoviruses (Plowright et al., 2008). Considering the anthropogenic pressures Amazonia is facing, one can suspect the emergence of new paramyxoviruses of unknown pathogenicity in humans (Calisher et al., 2006; Parrish et al., 2008; Moratelli and Calisher, 2015; Hayman, 2016; Allen et al., 2017; Johnson et al., 2020; Letko et al., 2020).

In conclusion, paramyxoviruses phylogenetically close to those reported from Brazil and Costa Rica were detected in *D. rotundus*, *C. perspicillata*, and *P. alitonus* in French Guiana, revealing a wide distribution of these variants at a continental scale. Their high prevalence in some species that can live in large multi-specific colonies increases the risk of transmission between species and the emergence of paramyxoviruses in humans. In addition, the paramyxoviruses described herein are close to viruses of the *Jeilongvirus* genus. Since some members of the *Jeilongvirus* genus are rodents, it would be interesting to look for paramyxoviruses in rodents in French Guiana. Better knowledge of paramyxovirus diversity in wild animals should improve our ability to predict changes in the dynamics of these viruses in the context of environmental perturbations.

Funding

This study was funded by the CAROLIA and RESERVOIRS programs supported by European Funds (ERDF/FEDER), the Région Guyane and the DDirection Régionale pour la Recherche et la Technologie. It also received "Investissement d'Avenir" grants managed by the Agence Nationale de la Recherche (CEBA, Ref. ANR-10-LABX-25-01). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors' contributions

ED and AL conceived the project, AL and BdT provided samples, ED and DD performed the laboratory work, ED, DD, and AL performed the sequence and phylogenetic analyses. ED, AL, and VL wrote the paper. All authors read and commented on the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgments

All field volunteers and owners and/or managers of capture sites are warmly acknowledged for their assistance in captures.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.meegid.2021.104769>.

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