



Taxonomic updates for the genus *Gyrovirus* (family *Anelloviridae*): recognition of several new members and establishment of species demarcation criteria

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Abstract

The genus *Gyrovirus* was assigned to the family *Anelloviridae* in 2017 with only one recognized species, *Chicken anemia virus*. Over the last decade, many diverse viruses related to chicken anemia virus have been identified but not classified. Here, we provide a framework for the classification of new species in the genus *Gyrovirus* and communicate the establishment of nine new species. We adopted the ‘Genus + freeform epithet’ binomial system for the naming of these species.

The genus *Gyrovirus* was assigned to the family *Anelloviridae* in 2017 [20] to better align with the genomic properties of the viruses in this family. Like other members of the family *Anelloviridae*, gyroviruses have negative-sense single-stranded DNA genomes that contain at least three large open reading frames. As *Chicken anemia virus* [8] has been the sole species in the genus *Gyrovirus*, there have been no established guidelines for the classification of a new species within this genus. However, over the last decade, the nucleotide sequences of 49 genomes of viruses related to chicken anemia virus (CAV) have been determined from

various species (*Elaphe carinata*, *Felis catus*, *Fulmarus glacialis*, *Gallus gallus*, *Homo sapiens*, *Hydrobates homochroa*, *Mustela putorius furo*, and *Myrmoderus ferrugineus*) (Table 1) but have remained unclassified [3–6, 9–11, 15–17, 19, 23, 29–32].

Here, we provide a framework for the classification of viruses in the genus *Gyrovirus*. We extracted the large open reading frame (ORF) VP1 [22] sequences of gyroviruses (n = 229) from full-genome sequences available in the GenBank database (including those of CAV, GenBank accessed on 6 July 2020) to determine the distribution of pairwise identity values using SDT v1.2 [14]. The plot of the distribution of the pairwise identity values (Fig. 1A) reveals a clear

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Table 1 Summary of the classification of new members of the genus *Gyrovirus*

New gyrovirus species	Accession no.	Exemplar virus for the species	Host	Country/region	Isolation source	Publication
<i>Gyrovirus fulgla1</i>	KR137527	Gyrovirus GyV8	<i>Fulmarus glacialis</i>	USA	Spleen and uropygial gland tissue	[10]
<i>Gyrovirus galga1</i>	HM590588	Avian gyrovirus 2	<i>Gallus gallus</i>	Brazil	Serum	[19]
	JQ690763	-	<i>Homo sapiens</i>	China	Feces	Unpublished
	KJ452213	-	<i>Mustela putorius furo</i>	Hungary	Feces	[5]
	KJ452214	-	<i>Mustela putorius furo</i>	Hungary	Feces	[5]
	KU168250	-	<i>Gallus gallus</i>	Italy	Serum	Unpublished
	KX708506	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KX708507	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KX708508	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KX708509	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KX708510	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KX708511	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KX708512	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KX708513	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KX708514	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KX708515	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KX708516	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KX708517	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KX708518	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KX708519	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KX708520	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KX708521	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KX708522	-	<i>Gallus gallus</i>	China	Liver; spleen	[31]
	KY039279	-	<i>Gallus gallus</i>	Brazil	Feces	[11]
	MG846492	-	<i>Gallus gallus</i>	Brazil	Feces	[11]
	MK089244	-	<i>Felis catus</i>	China	Feces	[15]
	MK089245	-	<i>Felis catus</i>	China	Feces	[15]
	MK089246	-	<i>Felis catus</i>	China	Feces	[15]
MK840982	-	<i>Elaphe carinata</i>	China	Liver	[30]	
MT671981	-	<i>Gallus gallus</i>	Brazil	muscle	[4]	
<i>Gyrovirus galga2</i>	KM111536	Gyrovirus GyV7-SF	<i>Gallus gallus</i>	USA	muscle	[32]
<i>Gyrovirus homsa1</i>	JQ308210	Gyrovirus GyV3	<i>Homo sapiens</i>	Chile	Feces	[16]
	KM348009	-	<i>Mustela putorius furo</i>	Hungary	Feces	[6]
	MG366592	-	<i>Gallus gallus</i>	China	Proventricular	[9]
	MK089247	-	<i>Felis catus</i>	China	Feces	[15]
	MK089248	-	<i>Felis catus</i>	China	Feces	[15]
	MK089249	-	<i>Felis catus</i>	China	Feces	[15]
	MT671982	-	<i>Gallus gallus</i>	Brazil	muscle	[4]
<i>Gyrovirus homsa2</i>	KF294862	Gyrovirus Tu789	<i>Homo sapiens</i>	Tunisia	Feces	[17]
	MK089250	-	<i>Felis catus</i>	China	Feces	[15]
	MK089251	-	<i>Felis catus</i>	China	Feces	[15]
<i>Gyrovirus homsa3</i>	JX310702	Gyrovirus 4	<i>Homo sapiens</i>	Hong Kong	Feces	[3]
	KJ452215	-	<i>Mustela putorius furo</i>	Hungary	Feces	[5]
	KY024580	-	<i>Gallus gallus</i>	Brazil	Feces	[11]
	MG846493	-	<i>Gallus gallus</i>	Brazil	Feces	[12]
	MT671983	-	<i>Gallus gallus</i>	Brazil	Muscle	[4]
<i>Gyrovirus homsa4</i>	KF294861	Gyrovirus Tu243	<i>Homo sapiens</i>	Tunisia	Feces	[17]

Table 1 (continued)

New gyrovirus species	Accession no.	Exemplar virus for the species	Host	Country/region	Isolation source	Publication
<i>Gyrovirus hydho1</i>	MH378452	Ashy storm petrel gyrovirus	<i>Hydrobates homochroa</i>	USA	Cloacal swab	[29]
<i>Gyrovirus myferr1</i>	MH638372	Gyrovirus 11	<i>Myrmoderus ferrugineus</i>	French Guiana	Cloacal swab	[23]

trough in the distribution between 67% and 92% pairwise identity. In a revision of the species demarcation criteria for members of the genus *Anellovirus* based on pairwise alignments (rather than global-alignment-derived pairwise identity scores) [28], we propose a species demarcation criterion of 69% based on the ORF1 coding open reading frame (ORF) of mammalian-infecting anelloviruses. To align species demarcation across the family *Anelloviridae*, we define a 69% VP1 nucleotide sequence pairwise identity threshold for species demarcation also for gyroviruses. Based on this, we establish nine new species to accommodate the 49 unclassified gyroviruses (Table 1). We provide further support for these species assignments using a colour-coded pairwise identity matrix and a midpoint-rooted maximum-likelihood phylogenetic tree of the VP1 nucleotide sequences (Fig. 1B and C).

For naming of the nine new species, we adopted the binomial “Genus + freeform epithet” species nomenclature outlined by Siddell *et al.* [21]. A summary of the 47 newly classified gyroviruses is provided in Table 1.

We recommend the following guidelines for establishing new species within the genus *Gyrovirus* to align with what has been recommended previously for classification of viruses in the families *Circoviridae* [20], *Geminiviridae* [2, 13, 24, 25], *Genomoviridae* [26], and *Smacoviridae* [27] as well as single-stranded DNA satellite molecules in the family *Alphasatellitidae* [1]:

1. If the complete VP1 ORF of a new gyrovirus has >69% nucleotide pairwise identity to that of any member

assigned to a currently classified gyrovirus species, the virus belongs to that particular species.

- a. In the event that the complete VP1 ORF of a new gyrovirus has >69% nucleotide pairwise identity to those of members of more than one gyrovirus species, the virus should be considered a member of the species with whose members it shares the highest percentage VP1 pairwise identity.
 - b. In the event that the complete VP1 ORF of a new gyrovirus has >69% nucleotide pairwise identity to those of one or more members assigned to a particular gyrovirus species, even if it shares <69% identity with those of the majority of the members assigned to that particular gyrovirus species, the virus should nevertheless be considered a member of that particular species.
2. If the complete VP1 ORF of a new gyrovirus has <69% nucleotide pairwise identity to those of all members of currently classified gyrovirus species, the virus should be considered a member of a new species.

We would like to highlight that different tools can yield different pairwise identity values, depending on how they are calculated. We recommend that true pairwise identity determination tools be used rather than those that infer pairwise identity values from multiple alignments. The latter generally tend to deflate the score due to gaps in the global multiple alignment and thus do not reflect the true pairwise identity between two sequences.

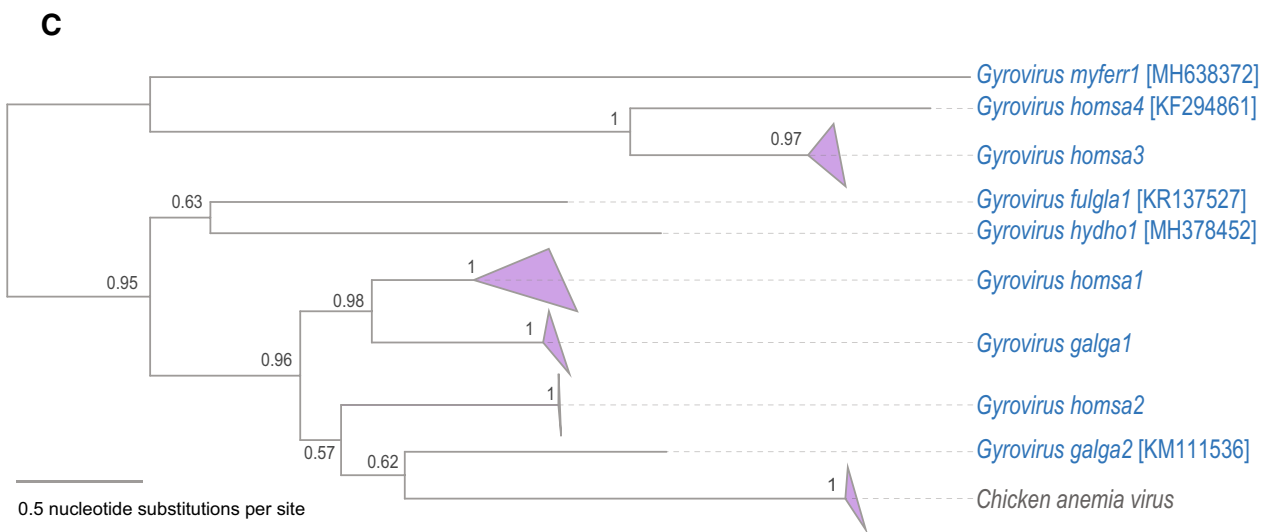
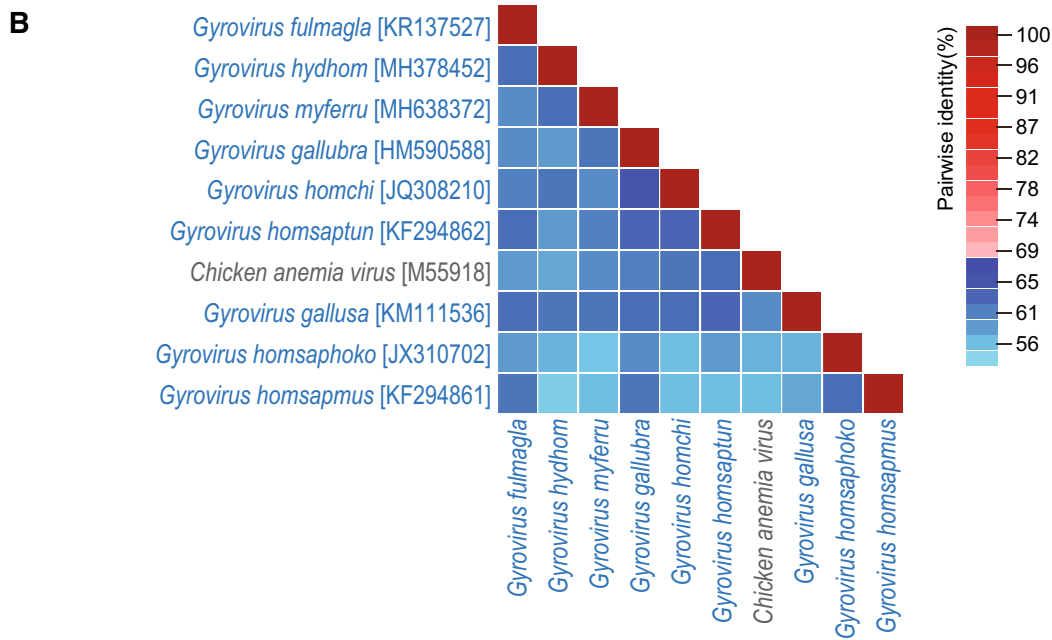
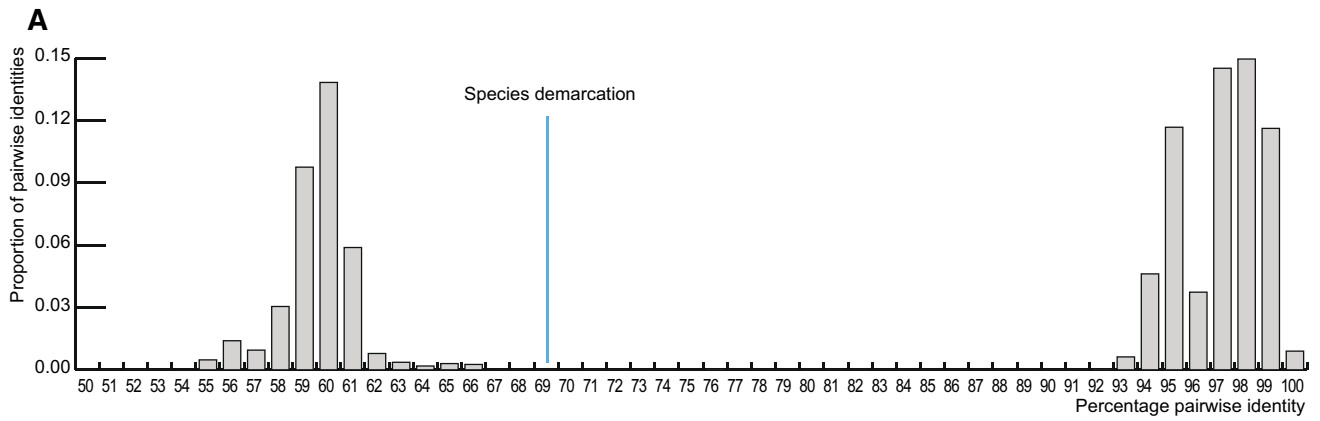


Fig. 1 **A** Distribution of pairwise identity values for VP1 nucleotide sequences of gyroviruses available in the GenBank database ($n = 229$). **B** Pairwise identity matrix of representative sequences from each species inferred using SDT v1.2 [14]. **C** Maximum-likelihood phylogenetic tree of the aligned VP1 sequences of representative gyroviruses inferred using PHYML [7] with the GTR+G model (determined using jModelTest) [18] and aLRT branch support. The tree is rooted at the midpoint. New species are shown in blue.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

References

- Bridson RW, Martin DP, Roumagnac P, Navas-Castillo J, Fiallo-Olive E, Moriones E, Lett JM, Zerbini FM, Varsani A (2018) Alphasatellitidae: a new family with two subfamilies for the classification of geminivirus- and nanovirus-associated alphasatellites. *Arch Virol* 163:2587–2600
- Brown JK, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva JC, Fiallo-Olive E, Bridson RW, Hernandez-Zepeda C, Idris A, Malathi VG, Martin DP, Rivera-Bustamante R, Ueda S, Varsani A (2015) Revision of Begomovirus taxonomy based on pairwise sequence comparisons. *Arch Virol* 160:1593–1619
- Chu DK, Poon LL, Chiu SS, Chan KH, Ng EM, Bauer I, Cheung TK, Ng IH, Guan Y, Wang D, Peiris JS (2012) Characterization of a novel gyrovirus in human stool and chicken meat. *J Clin Virol* 55:209–213
- Cibulski S, Alves de Lima D, Fernandes Dos Santos H, Teixeira TF, Tochetto C, Mayer FQ, Roehe PM (2021) A plate of viruses: Viral metagenomics of supermarket chicken, pork and beef from Brazil. *Virology* 552:1–9
- Feher E, Pazar P, Kovacs E, Farkas SL, Lengyel G, Jakab F, Martella V, Banyai K (2014) Molecular detection and characterization of human gyroviruses identified in the ferret fecal virome. *Arch Virol* 159:3401–3406
- Feher E, Pazar P, Lengyel G, Phan TG, Banyai K (2015) Sequence and phylogenetic analysis identifies a putative novel gyrovirus 3 genotype in ferret feces. *Virus Genes* 50:137–141
- Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* 59:307–321
- Kato A, Fujino M, Nakamura T, Ishihama A, Otaki Y (1995) Gene organization of chicken anemia virus. *Virology* 209:480–488
- Li G, Yuan S, He M, Zhao M, Hao X, Song M, Zhang L, Qiao C, Huang L, Zhang L, Li C, Wang G, Cheng Z (2018) Emergence of gyrovirus 3 in commercial broiler chickens with transmissible viral proventriculitis. *Transbound Emerg Dis* 65:1170–1174
- Li L, Pesavento PA, Gaynor AM, Duerr RS, Phan TG, Zhang W, Deng X, Delwart E (2015) A gyrovirus infecting a sea bird. *Arch Virol* 160:2105–2109
- Lima DA, Cibulski SP, Finkler F, Teixeira TF, Varela APM, Cerva C, Loiko MR, Scheffer CM, Dos Santos HF, Mayer FQ, Roehe PM (2017) Faecal virome of healthy chickens reveals a large diversity of the eukaryote viral community, including novel circular ssDNA viruses. *J Gen Virol* 98:690–703
- Lima DA, Cibulski SP, Tochetto C, Varela APM, Finkler F, Teixeira TF, Loiko MR, Cerva C, Junqueira DM, Mayer FQ, Roehe PM (2019) The intestinal virome of malabsorption syndrome-affected and unaffected broilers through shotgun metagenomics. *Virus Res* 261:9–20
- Muhire B, Martin DP, Brown JK, Navas-Castillo J, Moriones E, Zerbini FM, Rivera-Bustamante R, Malathi VG, Bridson RW, Varsani A (2013) A genome-wide pairwise-identity-based proposal for the classification of viruses in the genus Mastrevirus (family Geminiviridae). *Arch Virol* 158:1411–1424
- Muhire BM, Varsani A, Martin DP (2014) SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. *PLoS ONE* 9:e108277
- Niu JT, Yi SS, Dong GY, Guo YB, Zhao YL, Huang HL, Wang K, Hu GX, Dong H (2019) Genomic characterization of diverse gyroviruses identified in the feces of domestic cats. *Sci Rep* 9:13303
- Phan TG, Li L, O’Ryan MG, Cortes H, Mamani N, Bonkougou IJO, Wang C, Leutenegger CM, Delwart E (2012) A third gyrovirus species in human faeces. *J Gen Virol* 93:1356–1361
- Phan TG, Vo NP, Sdiri-Loulizi K, Aouni M, Pothier P, Ambert-Balay K, Deng X, Delwart E (2013) Divergent gyroviruses in the feces of Tunisian children. *Virology* 446:346–348
- Posada D (2008) jModelTest: phylogenetic model averaging. *Mol Biol Evol* 25:1253–1256
- Rijsewijk FA, Dos Santos HF, Teixeira TF, Cibulski SP, Varela AP, Dezen D, Franco AC, Roehe PM (2011) Discovery of a genome of a distant relative of chicken anemia virus reveals a new member of the genus Gyrovirus. *Arch Virol* 156:1097–1100
- Rosario K, Breitbart M, Harrach B, Segales J, Delwart E, Biagini P, Varsani A (2017) Revisiting the taxonomy of the family Circoviridae: establishment of the genus Cyclovirus and removal of the genus Gyrovirus. *Arch Virol* 162:1447–1463
- Siddell SG, Walker PJ, Lefkowitz EJ, Mushegian AR, Dutilh BE, Harrach B, Harrison RL, Junglen S, Knowles NJ, Kropinski AM, Krupovic M, Kuhn JH, Nibert ML, Rubino L, Sabanadzovic S, Simmonds P, Varsani A, Zerbini FM, Davison AJ (2020) Binomial nomenclature for virus species: a consultation. *Arch Virol* 165:519–525
- Todd D, Creelan JL, Mackie DP, Rixon F, McNulty MS (1990) Purification and biochemical characterization of chicken anaemia agent. *J Gen Virol* 71(Pt 4):819–823
- Truchado DA, Diaz-Piqueras JM, Gomez-Lucia E, Domenech A, Mila B, Perez-Tris J, Schmidt-Chanasit J, Cadar D, Benitez L (2019) A novel and divergent gyrovirus with unusual genomic features detected in wild passerine birds from a remote rainforest in French Guiana. *Viruses* 11:1148
- Varsani A, Martin DP, Navas-Castillo J, Moriones E, Hernandez-Zepeda C, Idris A, Murilo Zerbini F, Brown JK (2014) Revisiting the classification of circoviruses based on genome-wide pairwise identity. *Arch Virol* 159:1873–1882
- Varsani A, Navas-Castillo J, Moriones E, Hernandez-Zepeda C, Idris A, Brown JK, Murilo Zerbini F, Martin DP (2014) Establishment of three new genera in the family Geminiviridae: Becurtovirus, Eragrovirus and Turncurovirus. *Arch Virol* 159:2193–2203
- Varsani A, Krupovic M (2017) Sequence-based taxonomic framework for the classification of uncultured single-stranded DNA viruses of the family Genomoviridae. *Virus Evol* 3:vew037
- Varsani A, Krupovic M (2018) Smacoviridae: a new family of animal-associated single-stranded DNA viruses. *Arch Virol* 163:2005–2015
- Varsani A, Opriessnig T, Celer V, Maggi F, Okamoto H, Blomström A-L, Cadar D, Harrach B, Biagini P, Kraberger S (2021) Taxonomic update for mammalian anelloviruses (family Anelloviridae). *Arch Virol*. <https://doi.org/10.1007/s00705-021-05192-x>

29. Waits K, Bradley RW, Warzybok P, Krabberger S, Fontenele RS, Varsani A (2018) Genome Sequence of a Gyrovirus Associated with Ashy Storm-Petrel. *Microbiol Resour Announc* 7:e00958-18
30. Wu Q, Xu X, Chen Q, Ji J, Kan Y, Yao L, Xie Q (2019) Genetic analysis of avian gyrovirus 2 variant-related gyrovirus detected in farmed king ratsnake (*Elaphe carinata*): the first report from China. *Pathogens* 8:185
31. Yao S, Gao X, Tuo T, Han C, Gao Y, Qi X, Zhang Y, Liu C, Gao H, Wang Y, Wang X (2017) Novel characteristics of the avian gyrovirus 2 genome. *Sci Rep* 7:41068
32. Zhang W, Li L, Deng X, Kapusinszky B, Delwart E (2014) What is for dinner? Viral metagenomics of US store bought beef, pork, and chicken. *Virology* 468:303–310

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