2024 taxonomy update for the family *Circoviridae*

Arvind Varsani[1,2] · Balázs Harrach[3] · Philippe Roumagnac[4] · Mária Benkő[3] · Mya Breitbart[5] ·
Eric Delwart[6] · Giovanni Franzo[7] · Darius Kazlauskas[8,9] · Karyna Rosario[5] · Joaquim Segalés[10,11] ·
Emily Dunay[12] · Joshua Rukundo[13] · Tony L. Goldberg[12] · Enikő Fehér[3] · Eszter Kaszab[3] · Krisztián Bányai[3,14] ·
Mart Krupovic[15]

Abstract

Circovirids have a circular single-stranded DNA genome packed into a small icosahedral capsid. They are classified within two genera, *Circovirus* and *Cyclovirus*, in the family *Circoviridae* (phylum *Cressdnaviricota*, class *Arfiviricetes*, order *Cirlivirales*). Over the last five years, a number of new circovirids have been identified, and, as a result, 54 new species have been created for their classification based on the previously established species demarcation criterion, namely, that viruses classified into different species share less than 80% genome-wide pairwise sequence identity. Of note, one of the newly created species includes a circovirus that was identified in human hepatocytes and suspected of causing liver damage. Furthermore, to comply with binomial species nomenclature, all new and previously recognized species have been (re)named in binomial format with a freeform epithet. Here, we provide a summary of the properties of circovirid genomes and their classification as of June 2024 (65 species in the genus *Circovirus* and 90 species in the genus *Cyclovirus*). Finally, we provide reference datasets of the nucleotide and amino acid sequences representing each of the officially recognized circovirid species to facilitate further classification of newly discovered members of the *Circoviridae*.

✉ Arvind Varsani
arvind.varsani@asu.edu

✉ Mart Krupovic
mart.krupovic@pasteur.fr

1 The Biodesign Center for Fundamental and Applied Microbiomics, Center for Evolution and Medicine, School of Life Sciences, Arizona State University, Tempe, AZ 85287-5001, USA

2 Structural Biology Research Unit, Department of Integrative, Biomedical Sciences, University of Cape Town, Observatory, Cape Town 7925, South Africa

3 HUN-REN Veterinary Medical Research Institute, Budapest 1143, Hungary

4 CIRAD-UMR PHIM, Campus International de Baillarguet, 34398 Montpellier, France

5 College of Marine Science, University of South Florida, Saint Petersburg, FL 33701, USA

6 Department of Laboratory Medicine, University of California San Francisco, San Francisco, CA 94118, USA

7 Department of Animal Medicine, Production and Health (MAPS), Università di Padova, Viale dell'Università 16, 35020 Legnaro, Italy

8 Institute of Biotechnology, Life Sciences Center, Vilnius University, Saulétekio av. 7, 10257 Vilnius, Lithuania

9 National Center for Biotechnology Information, National Library of Medicine, NIH, Bethesda, MD 20894, USA

10 Departament de Sanitat i Anatomia Animals, Facultat de Veterinària, Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, 08193 Barcelona, Spain

11 Unitat Mixta d'Investigació IRTA-UAB en Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, 08193 Barcelona, Spain

12 Department of Pathobiological Sciences, University of Wisconsin-Madison School of Veterinary Medicine, Madison, WI, USA

13 Ngamba Island Chimpanzee Sanctuary/Chimpanzee Trust, Entebbe, Uganda

14 Department of Pharmacology and Toxicology, University of Veterinary Medicine, 1078 Budapest, Hungary

15 Institut Pasteur, Université Paris Cité, Archaeal Virology Unit, 25 rue du Dr Roux, 75015 Paris, France

Introduction

Circovirids have a small circular single-stranded DNA genome (~1670–2380 nt in length) that is encapsidated into a small icosahedral virion (20-25 nm) [1, 2]. All circovirids encode at least two proteins, the capsid protein (CP) and the replication-associated protein (Rep). The two proteins are encoded in an ambisense orientation [3], with the open reading frames (ORFs) located on the virion and complementary strands of the replicative form of the genome, respectively. The intergenic region between the 5' ends of the two major ORFs has a stem-loop structure with a conserved nonanucleotide sequence that serves as the origin of rolling-circle replication (RCR). The Reps of circovirids catalyze the initiation of RCR by nicking the viral DNA at the origin of replication, similar to the mechanism used by other cressdnaviricots [4, 5]. Circovirid Reps consist of two domains, the N-terminal HUH superfamily endonuclease domain and the C-terminal superfamily 3 helicase domain, each of which is characterized by conserved diagnostic motifs. Circovirid CPs consist of a positively charged N-terminal arm followed by a jelly-roll fold domain. Sixty copies of the CP assemble with $T = 1$ icosahedral symmetry to form isometric capsids [1, 6].

Circovirids are classified into two genera, *Circovirus* and *Cyclovirus* [7], within the family *Circoviridae* (phylum *Cressdnaviricota,* class *Arfiviricetes*, order *Cirlivirales*) [5]. A large part of what is known about circovirid biology and transmission is based on studies of circoviruses associated with psittacine beak and feather disease in parrots, runting syndrome in geese, young pigeon disease syndrome in pigeons, feather disorders in young ducks, and porcine circovirus diseases in pigs [8–11]. Recently, a human circovirus assumed to cause hepatitis in an immunosuppressed patient and intravenous drug users was reported [12, 13]. More generally, members of the genus *Circovirus* have been found to infect vertebrates, whereas those of the genus *Cyclovirus* are associated with both vertebrate and invertebrate hosts, but no robust virus-host systems have been reported yet.

The primary feature distinguishing circoviruses from cycloviruses is the orientation of the Rep and CP coding genes relative to the origin of replication. Furthermore, the Reps and CPs of circoviruses and cycloviruses are phylogenetically distinct. The same species demarcation thresholds are used for both genera, with viruses classified into separate species if their genomes share less than 80% genome-wide pairwise sequence identity with the classified family members [3].

Taxonomy update

In order to comply with the mandated binomial species naming format [14, 15], we have renamed the established species (n = 101, as of April 2023) in the family *Circoviridae* using binomial nomenclature with a freeform epithet. All epithets are derived from either the host species or the isolation source, using names in different languages of the peoples that inhabit the region of virus origin, whenever possible (Table 1), with the aim of creating a more inclusive virus nomenclature. In cases where hosts are known and the viruses were studied experimentally, and in order to have minimal disruption, we have used common names for the hosts. In the case of the former species *Porcine circovirus 1, Porcine circovirus 2, Porcine circovirus 3*, and *Porcine circovirus 4*, we have additionally used an alphanumeric epithet to rename them as *Circovirus porcine1*, *Circovirus porcine2*, *Circovirus porcine3,* and *Circovirus porcine4*. Notes on species naming are provided in Table 1. We would like to emphasize that only species names have changed, whereas virus names remain unchanged, i.e., porcine circovirus 2 (PCV2), beak and feather disease virus (BFDV), etc. should still be referred to as such. This is because there is a clear distinction in virology between a species (a taxonomical category) and a virus (a physical entity) [16].

Recently, a significant number of circovirus and cyclovirus genomes have been identified in various organisms or their fecal samples. Based on genome-wide pairwise sequence comparisons coupled with phylogenetic analysis, 55 new species (16 in the genus *Circovirus* and 39 in the genus *Cyclovirus*) have been created to classify these new circovirids (summarized in Table 1). All pairwise identity scores for the representative members of the genera *Circovirus* and *Cyclovirus* were determined using SDTv1.2 [17].

The binomial species names and the new species have been accepted and ratified by the International Committee on Taxonomy of Viruses (ICTV) [18].

Overview of representative viruses from each species in the family *Circoviridae*

Genomes

The genomes of circoviruses representing different species generally have a small intergenic region between the 3' ends of the ORFs encoding Rep and CP, and very few (~6%) of their Rep coding regions have introns (Fig. 1). In contrast, the representative genomes of cycloviruses rarely have intergenic regions between the 3' termini of

Table 1 Summary of new species and new binomial species names for representative members of genera *Circovirus* and *Cyclovirus*

Binomial species name	Epithet notes	Previous species name	Accession no.	Virus name	Host /source	Country of first description	Reference
Circovirus ban	coyote in Tohono O'odham	-	OQ599924	banfec circovirus 2	*Canis latrans*	USA	[43]
Circovirus barbel	common name for host	*Barbel circovirus*	GU799606	barbel circovirus	*Barbus barbus*	Hungary	[44]
Circovirus bastao	bat in Portuguese	*Bat associated circovirus 4*	KT783484	Tadarida brasiliensis circovirus 1	*Tadarida brasiliensis*	Brazil	[45]
Circovirus bear	common name for host	*Bear circovirus*	MN371255	Ursus americanus circovirus	*Ursus americanus americanus*	USA	[46]
Circovirus bianfu	bat in Chinese	*Bat associated circovirus 3*	JQ814849	Rhinolophus ferrumequinum circovirus 1	*Rhinolophus ferrumequinum*	China	[47]
Circovirus canary	common name for host	*Canary circovirus*	AJ301633	canary circovirus	*Serinus canaria*	Italy	[48]
Circovirus canine	common name for host	*Canine circovirus*	KC241982	canine circovirus	*Canis lupus familiaris*	USA	[49]
Circovirus catfish	common name for host	*European catfish circovirus*	JQ011377	Silurus glanis circovirus	*Silurus glanis*	Hungary	[50]
Circovirus chauvesouris	bat in French (2 words)	*Bat associated circovirus 1*	JX863737	bat associated circovirus 1	*Rhinolophus ferrumequinum*	Myanmar	[51]
Circovirus cia	bat in Lao	*Bat associated circovirus 12*	KJ641716	bat circovirus CV/GD2012	*Pipistrellus sp.*	China	[52]
Circovirus civet	common name for host	*Civet circovirus*	LC416389	Paguma larvata circovirus	*Paguma larvata*	Japan	[53]
Circovirus daga	rodent in Filipino	*Rodent associated circovirus 6*	KY370037	rodent circovirus 6	*Apodemus draco*	China	[54]
Circovirus duck	common name for host	*Duck circovirus*	AY228555	mulard duck circovirus	*Anas domesticus*	USA	[55]
Circovirus elk	common name for host	*Elk circovirus*	MN585201	elk circovirus	*Cervus canadensis*	Canada	[56]
Circovirus eniyan	human in Yoruba	*Human associated circovirus 1*	GQ404856	human stool-associated circular virus	*Homo sapiens*	Nigeria	[57]
Circovirus finch	common name for host	*Finch circovirus*	DQ845075	finch circovirus	*Chloebia gouldiae*	-	[33]
Circovirus gloton	wolverine in Spanish	-	MW686208	wolvfec circovius	*Gulo gulo*	USA	[58]
Circovirus gnaver	rodent in Danish	*Rodent associated circovirus 5*	KY370027	rodent circovirus 5	*Niviventer eha*	China	[54]
Circovirus goose	common name for host	*Goose circovirus*	AJ304456	goose circovirus	Goose	Germany	[59]
Circovirus gryzon	rodent in Polish	*Rodent associated circovirus 7*	MF497827	bamboo rat circovirus	*Rhizomys pruinosus*	China	-
Circovirus gull	common name for host	*Gull circovirus*	DQ845074	gull circovirus	*Larus argentatus*	Sweden	[33]
Circovirus gyurgyalag	bee-eater in Hungarian	-	MZ710935	bee-eater circovirus	*Merops apiaster*	Hungary	[60]
Circovirus hirat	rat in Volapuk	-	OM869597	dipodfec virus UA04Rod_4537	*Dipodomys merriami feces*	USA	[61]
Circovirus human	virus identified in human tissue	-	ON677309	human circovirus 1	*Homo sapiens*	France	[12]

Table 1 (continued)

Binomial species name	Epithet notes	Previous species name	Accession no.	Virus name	Host /source	Country of first description	Reference
Circovirus ialtag	bat in Scottish	*Bat associated circovirus 10*	KX756986	bat circovirus Acheng30	*Vespertilio sinensis*	China	[62]
Circovirus impundu	chimpanzee in Kinyarwanda	*Chimpanzee associated circovirus 1*	GQ404851	chimpanzee stool avian-like circovirus	*Pan troglodytes*	Rwanda	[57]
Circovirus kelawar	bat in Malay	*Bat associated circovirus 9*	KJ641741	bat associated circovirus 9	*Rhinolophus ferrum-equinum*	China	[52]
Circovirus kiore	rodent in Māori	*Rodent associated circovirus 2*	KY370042	rodent circovirus 2	*Apodemus chevrieri*	China	[54]
Circovirus kukwuria	coyote, scavenger in Comanche	-	OQ599922	banfec circovirus 1	*Canis latrans*	USA	[43]
Circovirus lepakko	bat in Finnish	*Bat associated circovirus 7*	KJ641723	bat associated circovirus 7	*Rhinolophus sinicus*	China	[52]
Circovirus lin	horse in Navajo	-	MW881235	equine circovirus 1	*Equus ferus caballus*	USA	[63]
Circovirus magu	mongoose in Punjabi	-	MZ382570	mongoose-associated circovirus Mon-1	*Urva auropunctata*	Saint Kitts and Nevis	[64]
Circovirus mink	common name for host	*Mink circovirus*	KJ020099	mink circovirus	*Mustela* sp.	China	[65]
Circovirus miztli	mountain lion in Nahuatl	-	MT610105	sonfela circovirus 1	*Lynx rufus*	Mexico	[66]
Circovirus miztontli	cat in Milpa Alta	-	MT610106	sonfela circovirus 2	*Lynx rufus*	Mexico	[66]
Circovirus morcego	bat in Galician	*Bat associated circovirus 8*	KJ641711	bat associated circovirus 8	*Myotis ricketti*	China	[52]
Circovirus mossi	common word for mosquito in USA	*Mosquito associated circovirus 1*	MH188038	Culex circovirus-like virus	*Culex* sp.	USA	[67]
Circovirus naaleeli	waterfowl in Navajo	-	MZ604582	wigfec circovirus 1	*Mareca americana*	USA	[68]
Circovirus parrot	common name for host	*Beak and feather disease virus*	AF071878	beak and feather disease virus	*Psittaciformes species*	USA	[35]
Circovirus pato	waterfowl in Spanish	-	MZ604590	wigfec circovirus 2	*Mareca americana*	USA	[68]
Circovirus penguin	common name for host	*Penguin circovirus*	MN164703	penguin circovirus	*Pygoscelis adeliae*	Antarctica	[37]
Circovirus pichong	tick in Chinese (two words Pí chóng)	*Tick associated circovirus 2*	KX987146	tick circovirus 2	*Ixodes crenulatus*	China	[69]
Circovirus pigeon	common name for host	*Pigeon circovirus*	AF252610	columbid circovirus	*Columbia livia*	Germany	[70]
Circovirus pipistrello	bat in Italian	*Bat associated circovirus 13*	MN928506	bat circovirus Sardinia	*Miniopterus schreibersii*	Italy	[71]
Circovirus porcine1	common name for host	*Porcine circovirus 1*	AF071879	porcine circovirus 1	*Sus scrofa domesticus*	Germany	[35]
Circovirus porcine2	common name for host	*Porcine circovirus 2*	AY651850	porcine circovirus 2	*Sus scrofa domesticus*	Canada	[72]
Circovirus porcine3	common name for host	*Porcine circovirus 3*	KT869077	porcine circovirus 3	*Sus scrofa domesticus*	USA	[73]
Circovirus porcine4	common name for host	*Porcine circovirus 4*	MK986820	porcine circovirus 4	*Sus scrofa domesticus*	China	[74]

Table 1 (continued)

Binomial species name	Epithet notes	Previous species name	Accession no.	Virus name	Host /source	Country of first description	Reference
Circovirus ratpenat	bat in Catalan	*Bat associated circovirus 6*	KJ641724	bat associated circovirus 6	*Rhinolophus affinis*	China	[52]
Circovirus raven	common name for host	*Raven circovirus*	DQ146997	raven circovirus	*Corvus coronoides*	Australia	[34]
Circovirus roditore	rodent in Italian	*Rodent associated circovirus 3*	KY370039	rodent circovirus 3	*Neodon clarkei*	China	[54]
Circovirus rongeur	rodent in French	*Rodent associated circovirus 1*	KY370034	rodent circovirus 1	*Neodon clarkei*	China	[54]
Circovirus rosegador	rodent in Catalan	*Rodent associated circovirus 4*	KY370029	rodent circovirus 4	*Allactaga sibirica*	China	[54]
Circovirus saguzarra	bat in Basque	*Bat associated circovirus 5*	KJ641727	bat associated circovirus 5	*Plecotus auritus*	China	[52]
Circovirus siksparnis	bat in Latvian	*Bat associated circovirus 11*	KX756996	bat circovirus Mengyuan2	*Hipposideros armiger*	China	[75]
Circovirus starling	common name for host	*Starling circovirus*	DQ172906	starling circovirus	*Sturnus vulgaris*	Spain	[76]
Circovirus swan	common name for host	*Swan circovirus*	EU056309	Cygnus olor circovirus	*Cygnus olor*	Germany	[77]
Circovirus tetning	seal in Norwegian	-	MN164712	werosea circovirus	*Leptonychotes weddellii*	Antarctica	[78]
Circovirus torpegem	little bittern in Hungarian	-	MZ710934	little bittern circovirus	*Ixobrychus minutus*	Hungary	[60]
Circovirus tzinaka	bat in Nahuatl	-	OL704833	Eumops bonariensis associated circovirus 1	*Eumops bonariensis*	Argentina	[79]
Circovirus vleermuis	bat in Dutch	*Bat associated circovirus 2*	KC339249	bat associated circovirus 2	*Rhinolophus ferrumequinum*	Myanmar	[51]
Circovirus wesa	cat in Cherokee	-	ON596197	calfel virus LSF45_cir359	*Lynx rufus*	USA	[40]
Circovirus whale	common name for host	*Whale circovirus*	MN103538	beaked whale circovirus	*Indopacetus pacificus*	USA	[80]
Circovirus yaa	tick in Navajo	*Tick associated circovirus 1*	KU230452	avian-like circovirus	*Ixodes scapulari*	USA	[81]
Circovirus zebrafinch	common name for host	*Zebra finch circovirus*	KP793918	zebra finch circovirus	*Taeniopygia guttata*	Germany	[82]
Cyclovirus aasiak	spider in Inuktitut	*Spider associated cyclovirus 1*	MH545516	soft spider associated circular virus 1	*Cybaeidae*	Canada	[83]
Cyclovirus adie	chicken in Yoruba	*Chicken associated cyclovirus 1*	HQ738643	cyclovirus NGchicken8/NGA/2009	*Gallus gallus*	Nigeria	[84]
Cyclovirus anyidiwo	ant in Ewe	-	ON324071	army ant associated cyclovirus 2 P8A-4.2_2	*Dorylus sp.*	Gabon	[42]
Cyclovirus babka	dragonfly in Ukrainian	*Dragonfly associated cyclovirus 6*	KC512918	dragonfly cyclovirus 6	*Aeshna multicolor*	USA	[85]
Cyclovirus bakri	goat in Urdu	*Goat associated cyclovirus 1*	HQ738636	cyclovirus PKgoat11/PAK/2009	*Capra aegagrus hircus*	Pakistan	[84]

Table 1 (continued)

Binomial species name	Epithet notes	Previous species name	Accession no.	Virus name	Host /source	Country of first description	Reference
Cyclovirus bashri	human in Arabic	*Human associated cyclovirus 4*	GQ404857	cyclovirus TN25	*Homo sapiens*	Tunisia	[57]
Cyclovirus bastao	bat in Portuguese	*Bat associated cyclovirus 10*	KM382270	bat circovirus POA/2012/VI	*Molossus molossus, Tadarida brasiliensis*	Brazil	[86]
Cyclovirus bohloa	ant in Sesotho	-	ON324104	army ant associated cyclovirus 5 170_4	*Dorylus sp.*	Gabon	[42]
Cyclovirus caballo	horse in Spanish	*Horse associated cyclovirus 1*	KR902499	cyclovirus Equ1	*Equus caballus*	USA	[87]
Cyclovirus cervienka	robin in Slovak	-	MZ350969	robinz virus RP_736	*Petroica australis*	New Zealand	[41]
Cyclovirus doi	bat in Vietnamese (originally two words)	*Bat associated cyclovirus 12*	KM382269	bat circovirus POA/2012/II	*Molossus molossus, Tadarida brasiliensis*	Brazil	[86]
Cyclovirus enseenene	ant in Luganda	-	ON324107	army ant associated cyclovirus 8 P1A-reste_2	*Dorylus sp.*	Gabon	[42]
Cyclovirus ezzike	chimpanzee in Luganda	-	OP934204	chimpanzee associated cyclovirus 2	*Pan troglodytes*	Uganda	[88]
Cyclovirus flagermus	bat in Danish	*Bat associated cyclovirus 5*	HQ738637	cyclovirus bat CyCV-TB	*Tadarida brasiliensis*	USA	[84]
Cyclovirus fledermoyz	bat in Yiddish	-	OM262456	chifec virus UA13_1880	*Tadarida brasiliensis*	USA	[89]
Cyclovirus foca	seal in Galician	-	MN176052	werosea cyclovirus	*Leptonychotes weddellii*	Antarctica	[78]
Cyclovirus fourmi	ant in French	-	ON324069	army ant associated cyclovirus 6 P16-reste_1	*Dorylus sp.*	Gabon	[42]
Cyclovirus gaaye	cow in Urdu	*Bovine associated cyclovirus 1*	HQ738634	cyclovirus PKbeef23/PAK/2009	*Bos taurus*	Pakistan	[84]
Cyclovirus gato	cat in Spanish	*Feline associated cyclovirus 1*	KM017740	feline cyclovirus	*Felis catus*	USA	[90]
Cyclovirus homa	human in Esperanto	*Human associated cyclovirus 5*	GQ404845	cyclovirus PK5034	*Homo sapiens*	Pakistan	[57]
Cyclovirus humana	human in Spanish	*Human associated cyclovirus 10*	KF726984	human associated cyclovirus 10	*Homo sapiens*	Chile	[91]
Cyclovirus ibimonyo	ant in Kinyarwanda	-	ON324066	army ant associated cyclovirus 3 P1A-reste_4	*Dorylus sp.*	Gabon	[42]
Cyclovirus insaan	human in Urdu	*Human associated cyclovirus 2*	GQ404844	cyclovirus PK500	*Homo sapiens*	Pakistan	[57]
Cyclovirus jaaabani	bat in Navajo	*Bat associated cyclovirus 1*	HM228874	bat cyclovirus GF-4c	*Antrozous pallidus*	USA	[92]
Cyclovirus jemage	bat in Hausa	*Bat associated cyclovirus 2*	JF938079	bat associated cyclovirus 2	*Myotis spp.*	China	[93]
Cyclovirus kacsa	duck in Hungarian	*Duck associated cyclovirus 1*	KY851116	duck associated cyclovirus 1	*Anas platyrhynchos*	Hungary	[94]

Table 1 (continued)

Binomial species name	Epithet notes	Previous species name	Accession no.	Virus name	Host /source	Country of first description	Reference
Cyclovirus kemirgen	rodent in Turkish	-	OM869613	dipodfec virus UA23Rod_1805	*Dipodomys spectabilis*	USA	[61]
Cyclovirus khangkhaw	bat in Norwegian	*Bat associated cyclovirus 9*	KJ641720	bat associated cyclovirus 9	*Tylonycteris pachypus*	China	[52]
Cyclovirus kiroptero	bat in Esperanto	*Bat associated cyclovirus 3*	JF938081	bat associated cyclovirus 3	*Myotis spp.*	China	[93]
Cyclovirus kisikisi	dragonfly in Tongan	*Dragonfly associated cyclovirus 1*	JX185419	dragonfly cyclovirus 1	*Pantala flavescens*	Tonga	[95]
Cyclovirus kokoro	ant in Yoruba	-	ON324100	army ant associated cyclovirus 1 P21/23-reste_1	*Dorylus sp.*	Gabon	[42]
Cyclovirus libelula	dragonfly in Spanish	*Dragonfly associated cyclovirus 5*	JX185426	dragonfly cyclovirus 5	*Erythrodiplax umbrata*	Puerto Rico	[95]
Cyclovirus liepsnele	robin in Lithuanian	-	MZ350973	robinz virus RP_1170	*Petroica australis*	New Zealand	[41]
Cyclovirus liliac	bat in Romanian	*Bat associated cyclovirus 7*	KJ641740	bat associated cyclovirus 7	*Rhinolophus pusillus*	China	[52]
Cyclovirus liljak	bat in Macedonian	-	OM262459	chifec virus UA15_2320	*Lasionycteris noctivagans*	USA	[96]
Cyclovirus maanav	human in Hindi	*Human associated cyclovirus 1*	GQ404847	cyclovirus PK5510	*Homo sapiens*	Pakistan	[57]
Cyclovirus manitan	human in Tamil	*Human associated cyclovirus 11*	KJ831064	cyclovirus SL-108277	*Homo sapiens*	Sri Lanka	[97]
Cyclovirus manukha	human in Punjabi	*Human associated cyclovirus 3*	GQ404846	cyclovirus PK5222	*Homo sapiens*	Pakistan	[57]
Cyclovirus manusyan	human in Malayalam	*Human associated cyclovirus 12*	KU053483	Indian encephalitis associated cyclovirus	*Homo sapiens*	India	-
Cyclovirus mchwa	ant in Swahili	*Ant associated cyclovirus 1*	MH545511	arboreal ant associated circular virus 1	*Crematogaster nigriceps*	Kenya	[83]
Cyclovirus mier	ant in Dutch	-	ON324103	army ant associated cyclovirus 4 P8A-3.2_1	*Dorylus sp.*	Gabon	[42]
Cyclovirus misi	cat in Quechua	-	ON596195	calfel virus LSF31_cyc420	*Lynx rufus*	USA	[40]
Cyclovirus mmadu	human in Igbo	*Human associated cyclovirus 6*	GQ404854	cyclovirus NG12	*Homo sapiens*	Nigeria	[57]
Cyclovirus moosa	cat in Hopi	-	ON596196	calfel virus LSF31_cyc880	*Lynx rufus*	USA	[40]
Cyclovirus munthu	human in Chichewa	*Human associated cyclovirus 9*	KC771281	human cyclovirus VS5700009	*Homo sapiens*	Malawi	[98]
Cyclovirus murcielago	bat in Spanish	-	OL704826	bat associated cyclovirus 17	*Molossus molossus*	Argentina	[79]
Cyclovirus muricec	bat in Catalan	-	OL704828	Eumops bonariensis associated cyclovirus 1	*Eumops bonariensis*	Argentina	[79]

Table 1 (continued)

Binomial species name	Epithet notes	Previous species name	Accession no.	Virus name	Host /source	Country of first description	Reference
Cyclovirus mutum	human in Hausa	*Human associated cyclovirus 7*	GQ404855	cyclovirus NG14	*Homo sapiens*	Nigeria	[57]
Cyclovirus mweyba	small Indian mongoose in Burmese	-	MZ382573	mongoose-associated cyclovirus Mon-20	*Urva auropunctata*	Saint Kitts and Nevis	[64]
Cyclovirus naahoohai	chicken in Navajo	*Chicken associated cyclovirus 2*	MG846358	chicken associated cyclovirus 2	*Gallus gallus*	Brazil	[99]
Cyclovirus naastsosi	small rodent in Navajo	*Mouse associated cyclovirus 1*	KT878836	mouse associated cyclovirus 1	mouse	USA	-
Cyclovirus nahkhiir	bat in Estonian	*Bat associated cyclovirus 8*	KJ641715	bat associated cyclovirus 8	*Rhinolophus pusillus*	China	[52]
Cyclovirus namu	dragonfly in Māori	*Dragonfly associated cyclovirus 7*	KC512919	dragonfly cyclovirus 7	*Xanthocnemis zealandica*	New Zealand	[85]
Cyclovirus ndanda	ant in Igbo	-	ON324108	army ant associated cyclovirus 9 183_1	*Dorylus* sp.	Gabon	[42]
Cyclovirus netopyr	bat in Czech	-	MT766312	bat associated cyclovirus Vr1	*Eptesicus regulus*	Australia	-
Cyclovirus newla	small Indian mongoose in Hindi	-	MZ382572	mongoose-associated cyclovirus Mon-32	*Urva auropunctata*	Saint Kitts and Nevis	[64]
Cyclovirus nhanloai	human in Vietnamese	*Human associated cyclovirus 8*	KF031466	cyclovirus VN	*Homo sapiens*	Vietnam	[100]
Cyclovirus nietoperz	bat in Polish	*Bat associated cyclovirus 13*	KJ641728	bat associated cyclovirus 13	*Plecotus auritus*	China	[52]
Cyclovirus pauferro	common word for this tree in Brazil - (pau ferro)	-	MT707947	Caesalpinia ferrea associated virus	*Libidibia ferrea*	Brazil	-
Cyclovirus pea	bat in Samoan	*Bat associated cyclovirus 15*	KT732786	Pacific flying fox associated cyclovirus-2	*Pteropus tonganus*	Tonga	[101]
Cyclovirus peka	bat in Tongan	*Bat associated cyclovirus 14*	KT732785	Pacific flying fox associated cyclovirus-1	*Pteropus tonganus*	Tonga	[101]
Cyclovirus pekapeka	bat in Māori	*Bat associated cyclovirus 16*	KT732787	Pacific flying fox associated cyclovirus-3	*Pteropus tonganus*	Tonga	[101]
Cyclovirus pettirosso	robin in Italian	-	MZ350965	robinz virus RP_493	*Petroica australis*	New Zealand	[41]
Cyclovirus podgana	rat in Slovenian	*Rodent associated cyclovirus 2*	KY370026	rodent associated cyclovirus 2	*Rattus tanezumi sladeni*	China	[54]
Cyclovirus popo	bat in Swahili	*Bat associated cyclovirus 6*	KJ641712	bat associated cyclovirus 6	*Rhinolophus pusillus*	China	[52]
Cyclovirus popoki	cat in Hawaiian	-	ON596192	calfel virus LSF17_cyc102	*Lynx rufus*	USA	[40]
Cyclovirus prihor	robin in Romanian	-	MZ350968	robinz virus RP_620	*Petroica australis*	New Zealand	[41]
Cyclovirus prilep	bat in Bulgarian	-	OM262453	chifec virus UA15_35	*Lasionycteris noctivagans*	USA	[96]

Table 1 (continued)

Binomial species name	Epithet notes	Previous species name	Accession no.	Virus name	Host /source	Country of first description	Reference
Cyclovirus punarinta	robin in Finnish	-	MZ350966	robinz virus RP_526	*Petroica australis*	New Zealand	[41]
Cyclovirus rata	rat in Galician	*Rodent associated cyclovirus 1*	KY370028	rodent associated cyclovirus 1	*Rattus flavipectus*	China	[54]
Cyclovirus risi	squirrel in Japanese	*Squirrel associated cyclovirus 1*	LC018134	cyclovirus TsCyV-1	*Callosciurus erythraeus thaiwanensis*	Japan	[102]
Cyclovirus roach	common name for cockroach in USA	*Cockroach associated cyclovirus 1*	JX569794	Florida woods cockroach cyclovirus	*Eurycotis floridana*	USA	[103]
Cyclovirus roedor	rodent in Portuguese	*Capybara associated cyclovirus*	MK947371	capybara associated cyclovirus 1	*Hydrochoerus hydrochaeris*	Brazil	[104]
Cyclovirus rotte	rat in Danish	-	OM869595	dipodfec virus UA04Rod_5913	*Dipodomys merriami*	USA	[61]
Cyclovirus rudzik	robin in Polish	-	MZ350967	robinz virus RP_584	*Petroica australis*	New Zealand	[41]
Cyclovirus saguza	bat in Basque	-	OM262451	chifec virus UA13_1727	*Tadarida brasiliensis*	USA	[89]
Cyclovirus sawya	bat in Hopi	-	OM262452	chifec virus UA13_1817	*Tadarida brasiliensis*	USA	[89]
Cyclovirus sismis	bat is Croatian	*Bat associated cyclovirus 4*	JF938082	bat associated cyclovirus 4	*Myotis spp.*	China	[93]
Cyclovirus sokwe	chimpanzee in Swahili	*Chimpanzee associated cyclovirus 1*	GQ404849	cyclovirus Chimp11	*Pan troglodytes*	Central Africa	[57]
Cyclovirus svosve	ant in Shona	-	ON324106	army ant associated cyclovirus 7 P4A-reste_1	*Dorylus sp.*	Gabon	[42]
Cyclovirus taniilai	dragonfly in Navajo	*Dragonfly associated cyclovirus 2*	JX185422	dragonfly cyclovirus 2	*Pantala flavescens*	USA	[95]
Cyclovirus tarako	dragonfly in Samoan	*Dragonfly associated cyclovirus 8*	KC512920	dragonfly cyclovirus 8	*Orthetrum Sabina*	Australia	[85]
Cyclovirus tonbo	dragonfly in Japanese	*Dragonfly associated cyclovirus 3*	JX185424	dragonfly cyclovirus 3	*Erythemis simplicicollis*	USA	[95]
Cyclovirus totoi	robin in Māori	-	MZ350964	robinz virus RP_259	*Petroica australis*	New Zealand	[41]
Cyclovirus vauval	bat in Tamil	-	MT766309	bat associated cyclovirus Cg1	*Chalinolobus gouldii*	Australia	-
Cyclovirus vazka	dragonfly in Slovakian	*Dragonfly associated cyclovirus 4*	KC512916	dragonfly cyclovirus 4	*Aeshna multicolor*	USA	[85]
Cyclovirus vespertilio	bat in Latin	-	OL704827	Tadarida brasiliensis associated cyclovirus 1	*Tadarida brasiliensis*	Argentina	[79]
Cyclovirus vleermuis	bat in Dutch	-	OM262457	chifec virus UA13_1887	*Tadarida brasiliensis*	USA	[89]
Cyclovirus yarasa	bat in Uzbek	*Bat associated cyclovirus 11*	KJ641717	bat associated cyclovirus 11	*Myotis spp.*	China	[52]
Cyclovirus ystlum	bat in Welsh	-	OM262458	chifec virus UA13_1800	*Tadarida brasiliensis*	USA	[89]

the ORFs encoding Rep and CP. Furthermore, a considerably larger fraction of cyclovirus ORFs have introns: ~33% of the Rep coding genes and one CP coding gene. The genome organization relative to the nucleotide motif in the stem-loop (involved in RCR) in cycloviruses is the mirror opposite of that of circoviruses (Fig. 2). Therefore, the reverse complement sequences of circovirus genomes are commonly used to root genome-based cyclovirus phylogenies and, conversely, the reverse complement sequences of cyclovirus genomes are used for the same purpose in circovirus phylogenies.

The distribution of genome sizes for representative circoviruses ranges from 1674 to 2224 nt (median: 1957 nt), whereas that of representative cycloviruses ranges from 1688 to 2379 nt (median: 1791.5 nt) (Fig. 3A). Notably, the % GC content (Fig. 3B) in the genomes of most cycloviruses is lower (median, 44.6; range: 34.6-52.1%) than that of circoviruses (median, 50.3; range: 42-56.2%), likely reflecting differences in the codon usage of their respective hosts. Consistent with the genome size, the Rep and CP coding regions of cycloviruses are smaller than those of circoviruses (Fig. 3C and D).

Conserved motifs

The nonanucleotide motifs located at the apex of the stem-loop, corresponding to the origin of replication, are very similar for circoviruses and cycloviruses, with variations observed only at positions 1 and 3 of the consensus sequence NANTATTAC (Fig. 4A and B).

Compared to other members of the *Cressdnaviricota*, all members of the *Circoviridae* display characteristic signatures within the conserved motifs of the HUH endonuclease and superfamily 3 helicase domains of their Reps. For instance, the conserved 'PP' in the Walker A motif is not common among other viruses [4]. In addition, circoviruses and cycloviruses display more-subtle, genus-specific variations within the three RCR motifs (motif I, II, and III) [19–21] and the superfamily 3 helicase motifs (Walker A, Walker B, motif C [22, 23] and the Arg finger [24]) (Fig. 4C and D).

Phylogenetic analysis of genome, Rep, and CP sequences

Maximum-likelihood phylogenetic trees were constructed based on genome sequences (Supplementary Data 1 and 2) as well as the Rep and CP amino acid sequences of representative members of each species of the genera *Circovirus* and *Cyclovirus*.

The trimmed genome sequence alignment-based maximum-likelihood phylogenetic trees of representative members of the genera *Circovirus* and *Cyclovirus* are shown in Figures 5 and 6, with the species names next to the corresponding accession numbers of the representative members. These phylogenetic trees illustrate the diversity of circovirids and provide insights into evolutionary relationships within both genera. Consistently, the genome-wide pairwise identity values obtained using SDT v1.2 [17] range from 54.94 to 79.37% and 54.25 to 78.85% for the representatives of the genera *Circovirus* and *Cyclovirus*, respectively (Fig. 7).

Two datasets of the Rep and CP amino acid sequences were assembled (Supplementary Data 4 and 5) and used for the downstream phylogenetic and comparative analyses. For the Rep dataset, we used three circovirus-like Rep sequences as an outgroup (AXH77637, AXQ03942, and ATP66722). For the CP dataset, the phylogenetic tree was rooted at the midpoint due to difficulties in aligning the CPs of circoviruses and cycloviruses reliably (Figs. 8 and 9). The Rep and CP phylogenetic trees support the species classification as well as the correct genus assignment for the various species-level taxa. Notably, based on pairwise identity values, the Rep is more conserved than the CP for all members of the family *Circoviridae* (Fig. 7), likely reflecting different selection pressures on these two proteins. The CP amino acid sequence identity values for representative circoviruses and cycloviruses range from 17.04 to 87.03% and from 12.62 to 72.69%, respectively.

In the supplementary information, we provide the reference datasets of genome sequences, Rep sequences, and CP nucleotide sequences representing each of the currently recognized species in the genera *Circovirus* (Supplementary Data 1, 3, 4) and *Cyclovirus* (Supplementary Data 2, 5, 6). We hope that these datasets will be useful to the community for further taxonomic assignments of new species.

Pitfalls of circovirid genome annotation

1) We encourage the community to carefully analyze the genome sequences of newly identified circovirids for the presence of introns in their genes. This will help both the researchers and the ICTV *Circoviridae* Study Group in classifying the corresponding viruses. We provide a fasta file of the genome sequences as well as the Rep and CP sequences as supplementary datasets in this article to help with intron identification and prediction of putative acceptor/donor sites. We direct the readers to Wright et al. [32] as a guide for detection of the intron 5' (GT) and 3' (AG) sequence boundaries in members of the family *Geminiviridae* (phylum *Cressdnaviricota*).

2) For convenience of comparison with reference circovirids, we recommend defining the first nucleotide of the
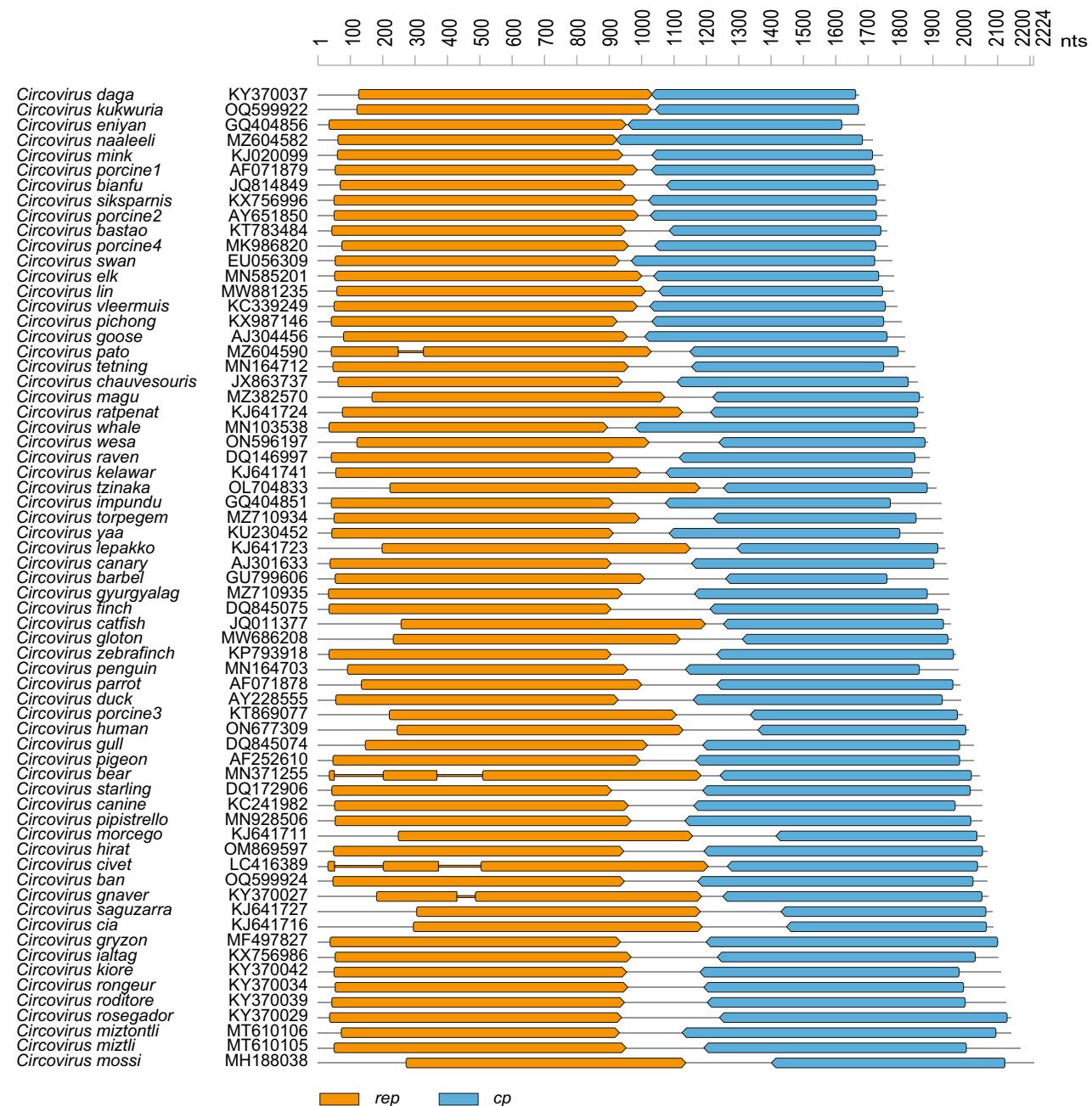
Fig. 1 Linearized representation of the genome organization of circoviruses representing each species. The binominal species names and the representative member accession numbers are provided to the left of each genome illustration. The Rep and CP coding genes are shown as orange and blue bars, respectively. Interruptions in the Rep regions correspond to introns.

nonanucleotide sequences at the *ori* site as position 1 of the genome. Thus, we urge researchers to identify the nonanucleotide motif and recircularize the genome sequence starting at this position prior to depositing it in GenBank or other public repositories.

3) In the cases of some circovirid CPs, there is evidence of an alternative start codon usage (e.g., serine as opposed to a methionine in avian circovirids belonging to the species *Circovirus gull* [33], *Circovirus raven* [34], *Circovirus parrot* [35], and *Circovirus penguin* [36, 37]). Thus, we encourage researchers to keep this alternative start codon used by circovirids in mind when analyzing open
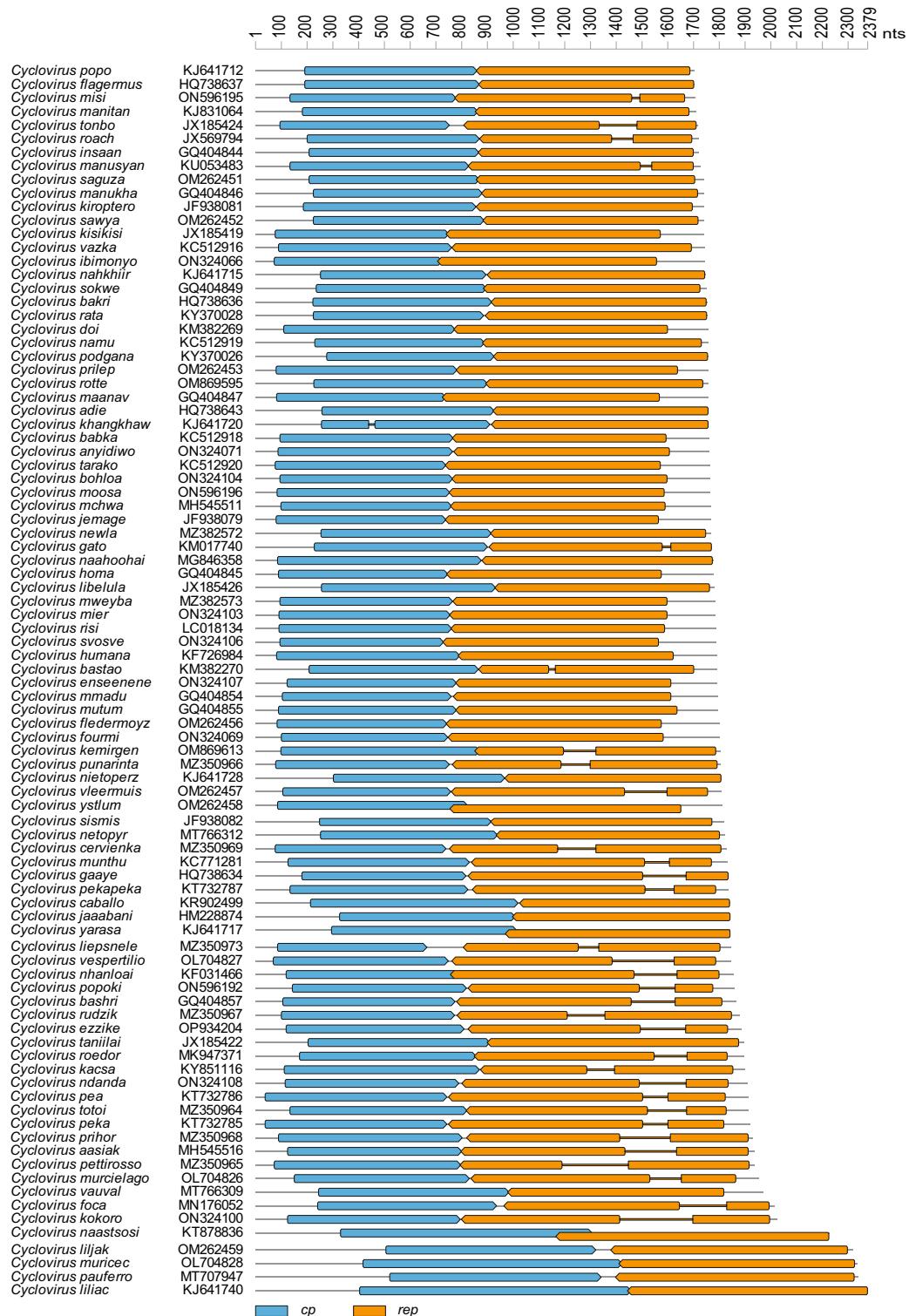
Fig. 2 Linearized representation of the genome organization of cycloviruses representing each species. The binominal species names and the representative member accession numbers are provided to the left of each genome illustration. The Rep and CP coding genes are shown in orange and blue bars, respectively. Interruptions in the Rep and CP regions correspond to introns.
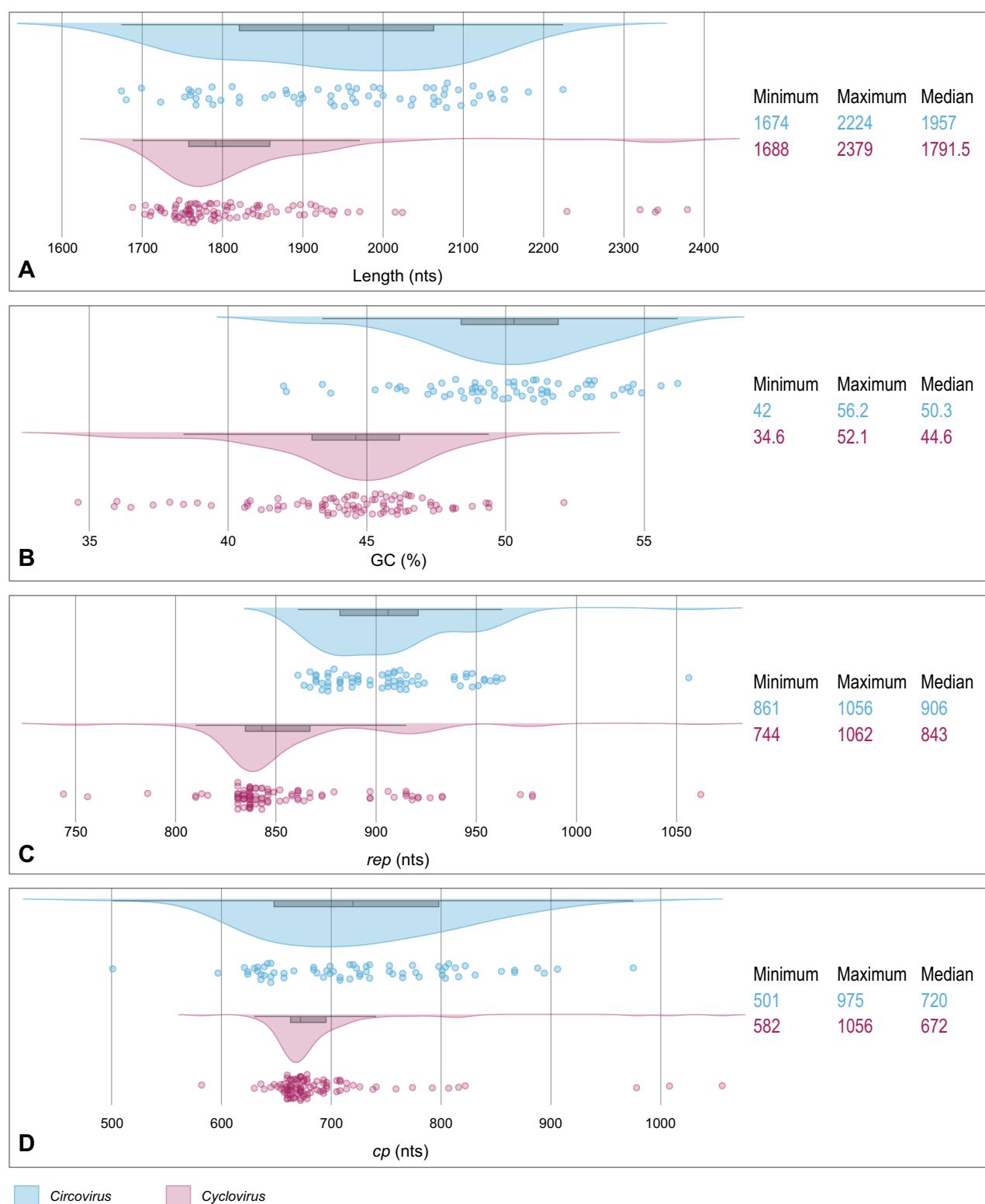
Fig. 3 Raincloud plots showing the distribution of genome lengths (A), % GC content of the genomes (B), the Rep coding gene length (C), and CP coding gene length (D) of representative members of the species in the genera *Circovirus* (blue) and *Cyclovirus* (purple)
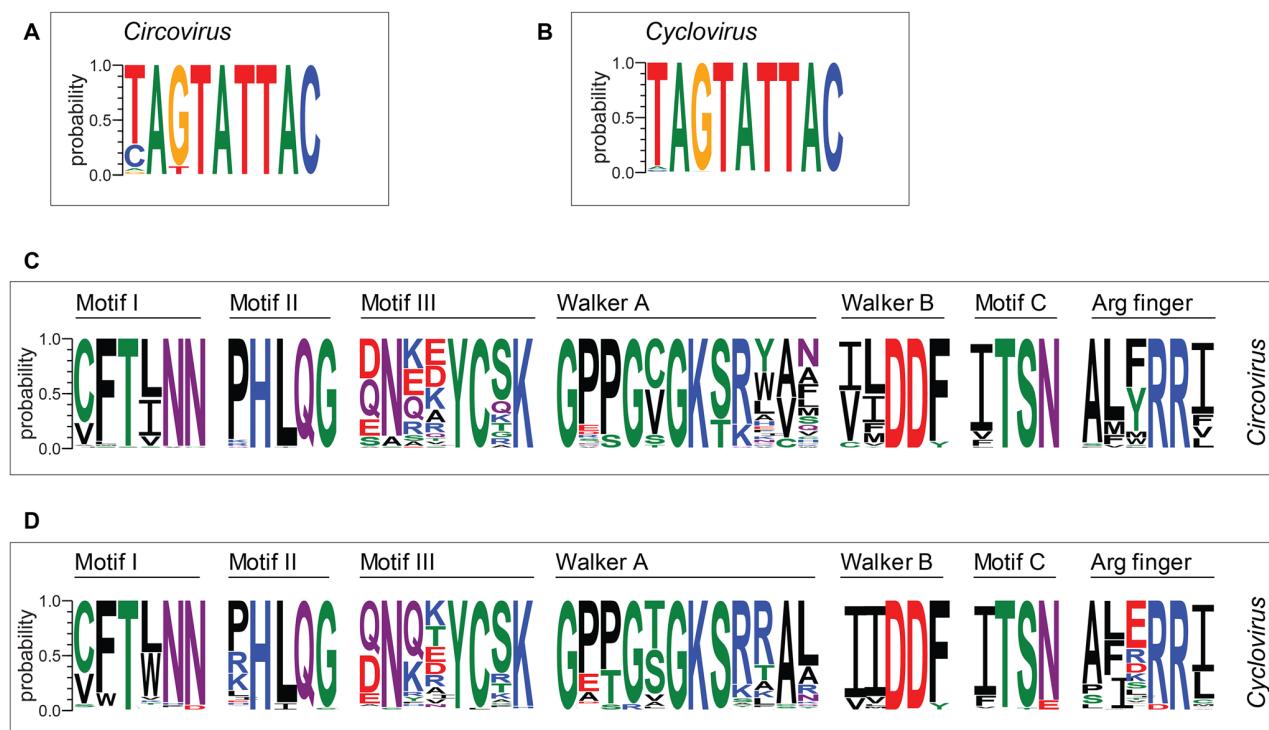
Fig. 4 The nonanucleotide motif sequences of members of the genera *Circovirus* (A) and *Cyclovirus* (B) depicted as sequence probability logos generated in Weblogo 3 [25]. (C and D) Sequence probability logos (generated using Weblogo 3 [25]) of the conserved amino acid residues characteristic of rolling-circle replication (RCR) endonu- cleases, including RCR motifs I, II, III, and superfamily 3 helicase motifs, including Walker A and B, motif C, and the Arg finger, found in replication-associated proteins (Rep) of members of the genera *Circovirus* (C) and *Cyclovirus* (D).

reading frames. It is also a good practice to align the CP coding region with those of reference circovirid CPs.

Recommendations for classification and nomenclature of viruses in the family *Circoviridae*

1) For species assignment and classification, please follow the guidelines established by Rosario et al. [3]. In brief, the species demarcation threshold for members of the family *Circoviridae* is 80% genome-wide pairwise iden- tity, i.e., only viruses sharing less than 80% identity with classified members of the family represent new species.

2) A large number of novel cressdnaviricots are being routinely identified in various ecosystems. Many of these viruses encode proteins homologous to those of circovirids but represent other viral groups/families in the phylum *Cressdnaviricota*. For instance, members of the family *Vilyaviridae* belong to the same order as the *Circoviridae* and encode similar Reps but neverthe- less represent a different virus family [38, 39]. Thus,

as a rough rule, as outlined by Rosario et al. [3], if a newly sequenced/identified virus genome shares <55% genome-wide pairwise identity with classified circo- virids, it is likely a member of a different family or an unclassified group of cressdnaviricots. We encour- age analysis of the Rep and CP phylogenies for a more informed assignment.

3) Note that there is a clear distinction between a species name and a virus name, as outlined by Zerbini et al. [16]. We urge correct use of virus names and species names.

   a. For example, the following statements are correct: "Viruses in the species *Circovirus porcine2* are associated with porcine circovirus-2-systemic dis- ease in pigs." or "Porcine circovirus 2 is a member of the species *Circovirus porcine2* and is the causa- tive agent of porcine circovirus-2-systemic disease in pigs."

   b. Incorrect use includes, for example, "*Circovirus porcine2* infects pigs and is associated with por- cine circovirus-2-systemic disease in pigs." Note that a virus species does not infect a host, because a

Fig. 5 Maximum-likelihood (ML) phylogenetic tree of representative members of each species in the genus *Circovirus* (Supplementary Data 1). The ML tree was inferred using PhyML 3 [26] with the GTR+I+G substitution model (determined using ModelTest2 [27]), using a trimmed (trimmed with TrimAl [28], gap threshold option of 0.2) alignment (aligned with MAFFT [29]) of the genome sequences of representative members of each species in the genus *Circovirus*. The tree is rooted with reverse complement sequences of representative cycloviruses (JX185419, KF726984, and KM017740), as their genomes are mirror opposite in terms of organization of Rep and CP coding genes. Branches with <80% SH-like support have been collapsed using TreeGraph2 [30].


0.2 nucleotide subs per site
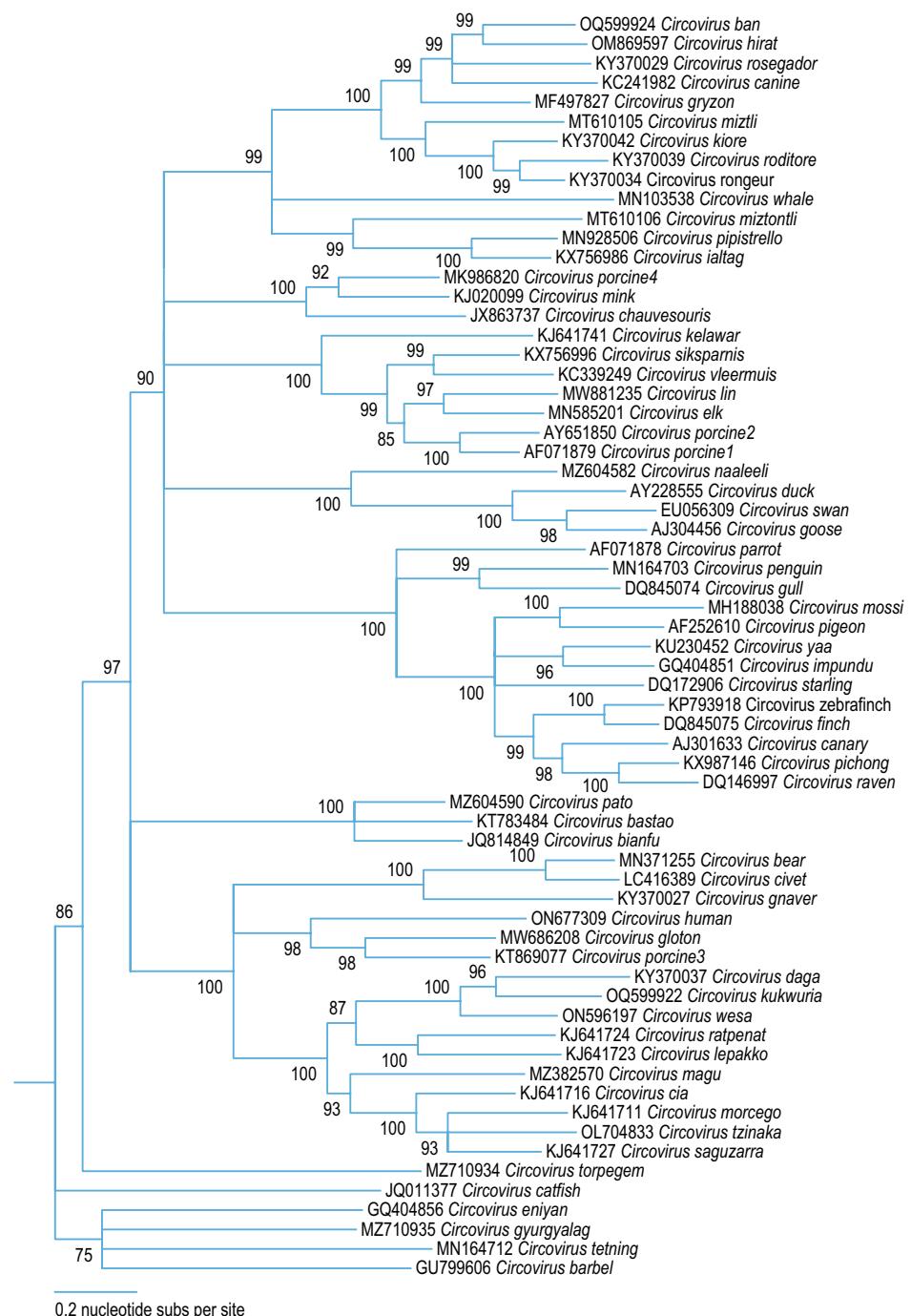
species is a taxonomic category and not a physical entity .

4) The ICTV *Circoviridae* Study Group evaluates new species annually, typically during the first quarter of the year, prior to assembling taxonomy proposals for review by the ICTV Executive Committee and the community at large. All members of the community are encouraged to submit taxonomy proposals to the ICTV (see https://ictv.global/).

5) Given that many circovirid genomes are sequenced by metagenomics of whole animals and environmental samples, in many cases, the true host of these viruses is unknown. To avoid confusion and issues with downstream data mining based on virus names, in the absence of robust evidence of host specificity, we encourage the

Fig. 6 Maximum-likelihood (ML) phylogenetic tree of representative members of each species in the genus *Cyclovirus* (Supplementary Data 2). The ML tree was inferred using PhyML 3 [26] with the GTR+I+G substitution model (determined using ModelTest2 [27]) using a trimmed (trimmed with TrimAl v1.4.1 [28], gap threshold option of 0.2) alignment (aligned with MAFFT [29]) of the genome sequences of representative members of each species in the genus *Cyclovirus*. The tree is rooted with reverse complement sequences of circoviruses (AF071878, AF071879, and JQ011377), as their genomes are mirror opposite in terms of organization of Rep and CP coding genes, Branches with <80% SH-like support have been collapsed using TreeGraph2 [30].

0.2 nucleotide subs per site
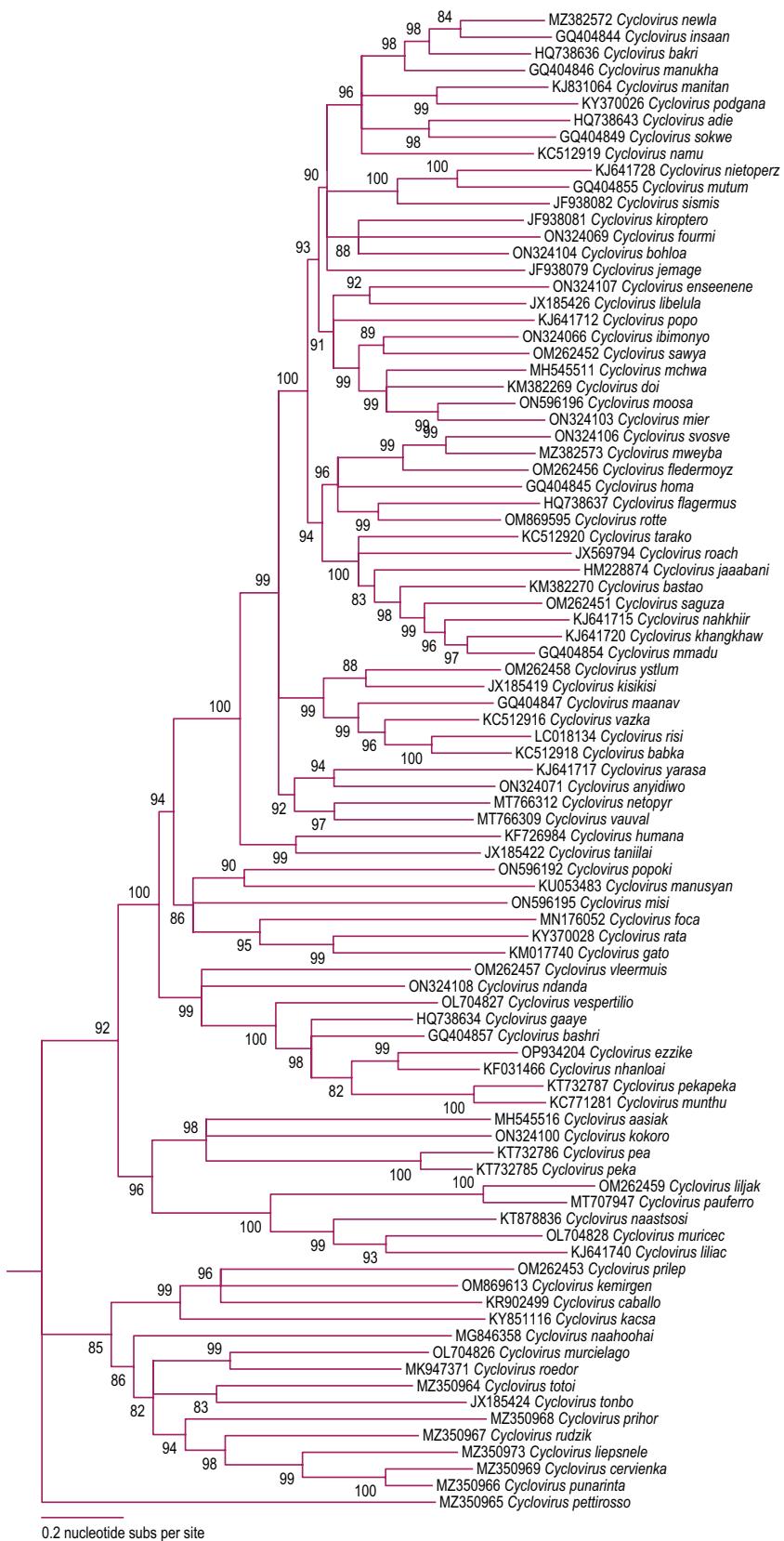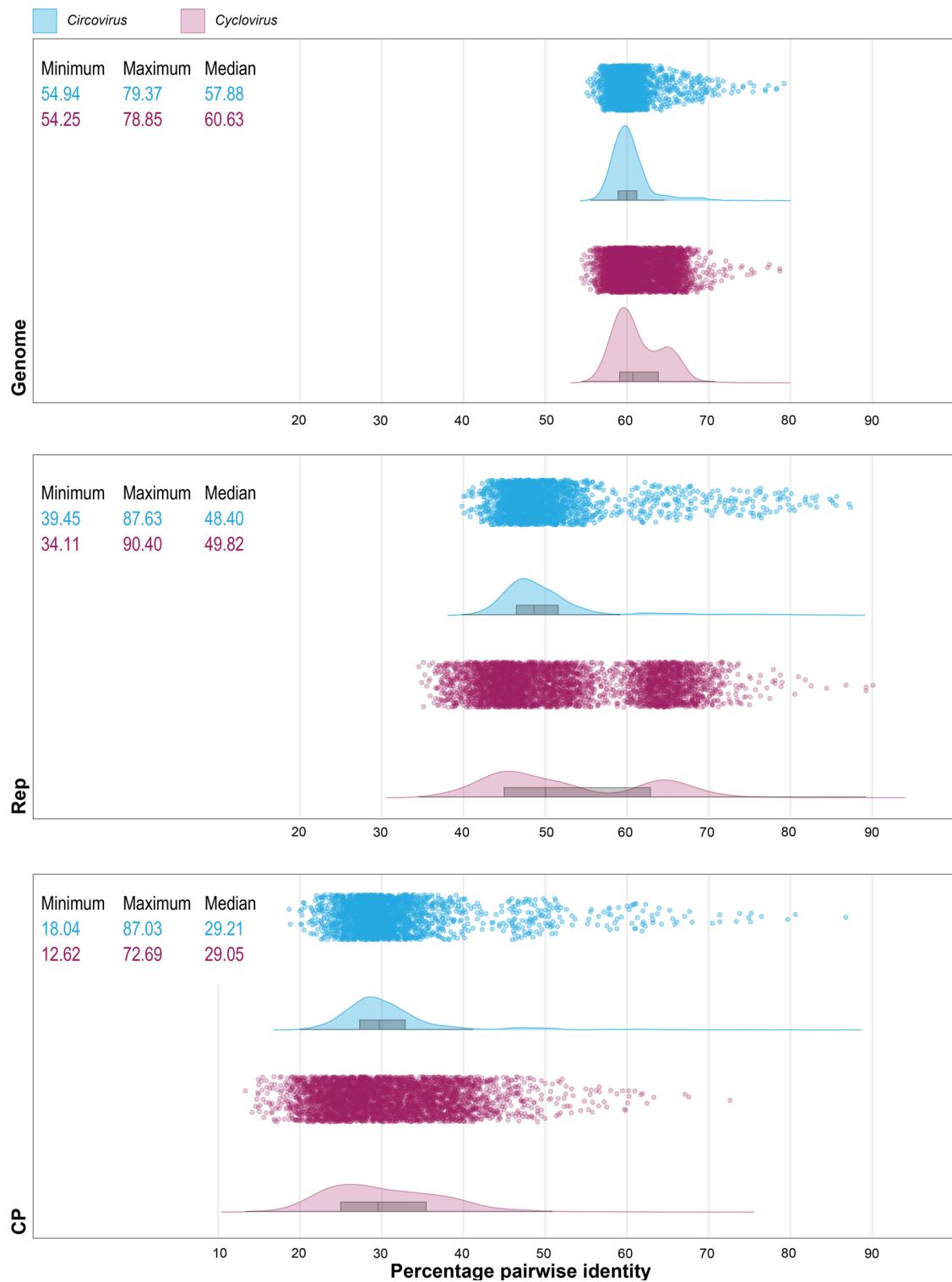
Fig. 7 Distribution of pairwise identity values, determined using SDTv1.2 [17], of the genome, Rep (amino acid) and CP (amino acid) sequences of representative members of each species in the genera *Circovirus* (blue) and *Cyclovirus* (purple)
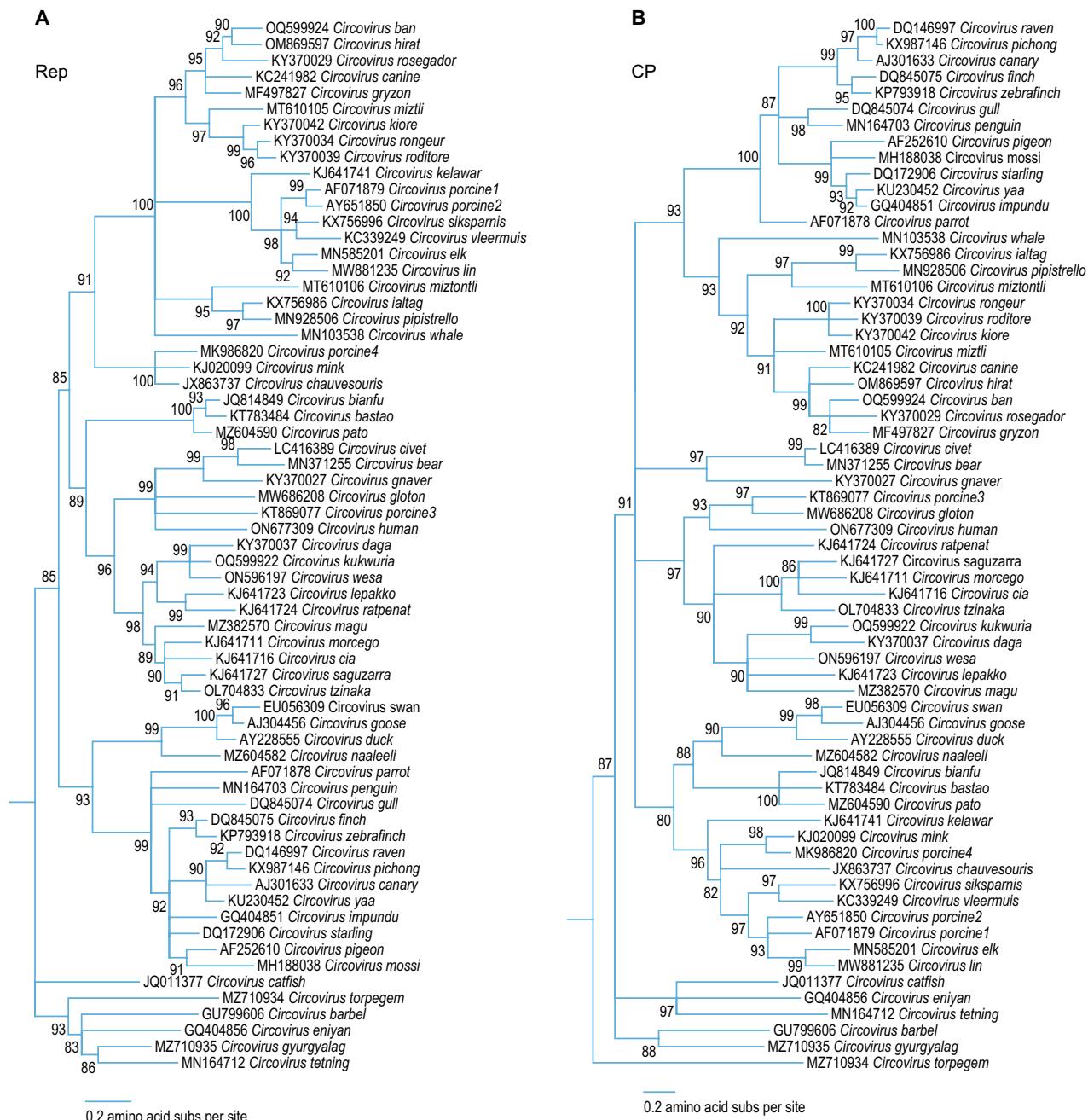
Fig. 8 Maximum-likelihood (ML) phylogenetic trees of the Rep (A) and CP (B) amino acid sequences of representative members of the genus *Circovirus*. The ML trees were inferred using PhyML 3 [26] with the RtREV+I+G and RtREV+G+F substitution model (inferred using ProtTest3 [31]) for the Rep and CP amino acid sequences, respectively, using trimmed (trimmed with TrimAl v1.4.1 [28], gap threshold option of 0.2) alignments (aligned with MAFFT [29]). Branches with <80% SH-like support have been collapsed using TreeGraph2 [30].

virology community to refrain from including suspected host names explicitly in the names of viruses. We provide the following examples for virus naming:

a. a circovirid identified in a fecal sample of a mountain lion from California was named calfel virus (derived from <u>Cal</u>ifornia <u>fel</u>id virus) [40];
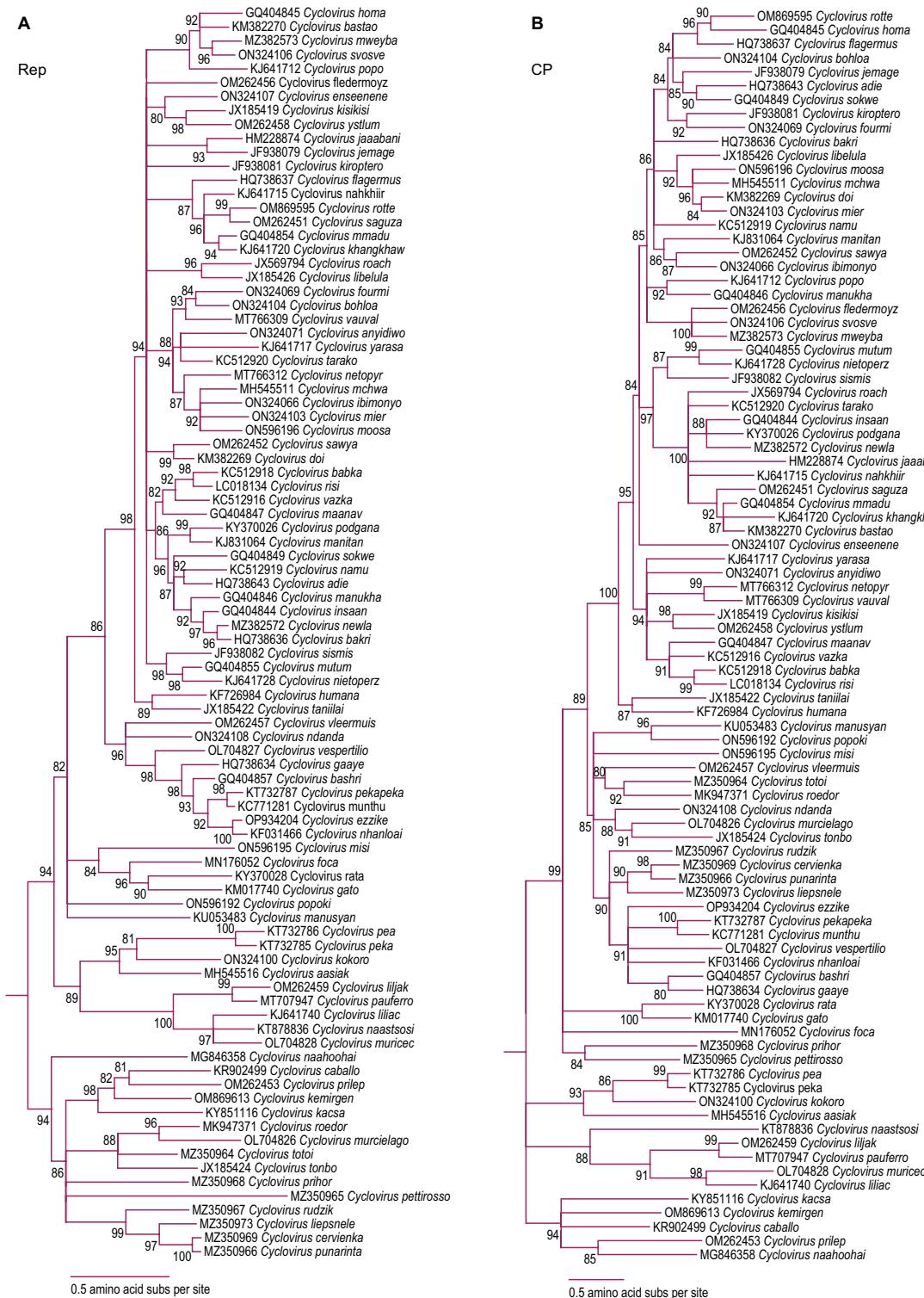
Fig. 9 Maximum-likelihood (ML) phylogenetic trees of the Rep (A) and CP (B) amino acid sequences of representative members of the genus *Cyclovirus*. The ML trees were inferred using PhyML 3 [26] with the RtREV+I+G and RtREV+G+F substitution model (inferred using ProtTest3 [31]) for Rep and CP amino acid sequences, respec-

tively, using trimmed (trimmed with TrimAl v1.4.1 [28], gap threshold option of 0.2) alignments (aligned with MAFFT [29]). Branches with <80% SH-like support have been collapsed using TreeGraph2 [30].

b. a circovirid identified in a fecal sample of a New Zealand black robin was named robinz virus (derived from <u>rob</u>in <u>N</u>ew <u>Z</u>ealand virus) [41];

c. a circovirid identified in army ant samples from Gabon was named army ant associated cyclovirus [42];

6) Prior to assigning the "circovirus" labels to newly discovered viruses, please verify that Reps and CPs of these viruses phylogenetically cluster with those of classified circovirids (Supplementary Data 3–6). Viruses wrongly labeled as "circoviruses" in GenBank create confusion and propagate naming errors.

7) Finally, we encourage the community also to review the Virus Metadata Resource (VMR; https://ictv.global/vmr) for metadata on species and representative members of each species.

Declarations

References

1. Khayat R, Brunn N, Speir JA, Hardham JM, Ankenbauer RG, Schneemann A, Johnson JE (2011) The 2.3-angstrom structure of porcine circovirus 2. J Virol 85:7856–7862

2. Crowther RA, Berriman JA, Curran WL, Allan GM, Todd D (2003) Comparison of the structures of three circoviruses: chicken anemia virus, porcine circovirus type 2, and beak and feather disease virus. J Virol 77:13036–13041

3. Rosario K, Breitbart M, Harrach B, Segales J, Delwart E, Biagini P, Varsani A (2017) Revisiting the taxonomy of the family *Circovirida*e: establishment of the genus *Cyclovirus* and removal of the genus *Gyrovirus*. Arch Virol 162:1447–1463

4. Kazlauskas D, Varsani A, Koonin EV, Krupovic M (2019) Multiple origins of prokaryotic and eukaryotic single-stranded DNA viruses from bacterial and archaeal plasmids. Nat Commun 10:3425

5. Krupovic M, Varsani A, Kazlauskas D, Breitbart M, Delwart E, Rosario K, Yutin N, Wolf YI, Harrach B, Zerbini FM, Dolja VV, Kuhn JH, Koonin EV (2020) *Cressdnaviricota*: a virus phylum unifying seven families of Rep-encoding viruses with single-stranded, circular DNA genomes. J Virol 94:e00582-20

6. Sarker S, Terron MC, Khandokar Y, Aragao D, Hardy JM, Radjainia M, Jimenez-Zaragoza M, de Pablo PJ, Coulibaly F, Luque D, Raidal SR, Forwood JK (2016) Structural insights into the assembly and regulation of distinct viral capsid complexes. Nat Commun 7:13014

7. Breitbart M, Delwart E, Rosario K, Segales J, Varsani A, Consortium IR (2017) ICTV Virus Taxonomy Profile: *Circoviridae*. J Gen Virol 98:1997-1998

8. Todd D (2000) Circoviruses: immunosuppressive threats to avian species: a review. Avian Pathol 29:373–394

9. Woods LW, Latimer KS, Niagro FD, Riddell C, Crowley AM, Anderson ML, Daft BM, Moore JD, Campagnoli RP, Nordhausen RW (1994) A retrospective study of circovirus infection in pigeons: nine cases (1986–1993). J Vet Diagn Invest 6:156–164

10. Harding JC (1998) Postweaning multisystemic wasting syndrome: epidemiology and clinical presentation. J Swine Health Prod 6:249–254

11. Segalés J, Domingo M (2002) Postweaning multisystemic wasting syndrome (PMWS) in pigs. A review. Vet Q 24:109–124

12. Pérot P, Fourgeaud J, Rouzaud C, Regnault B, Da Rocha N, Fontaine H, Le Pavec J, Dolidon S, Garzaro M, Chrétien D, Morcrette G, Molina TJ, Ferroni A, Leruez-Ville M, Lortholary O, Jamet A, Eloit M (2023) Circovirus Hepatitis Infection in Heart-Lung Transplant Patient, France. Emerg Infect Dis 29:286–293

13. Li Y, Zhang P, Ye M, Tian RR, Li N, Cao L, Ma Y, Liu FL, Zheng YT, Zhang C (2023) Novel Circovirus in Blood from Intravenous Drug Users, Yunnan, China. Emerg Infect Dis 29:1015–1019

14. 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

15. Walker PJ, Siddell SG, Lefkowitz EJ, Mushegian AR, Adriaenssens EM, Alfenas-Zerbini P, Davison AJ, Dempsey DM, Dutilh BE, Garcia ML, Harrach B, Harrison RL, Hendrickson RC, Junglen S, Knowles NJ, Krupovic M, Kuhn JH, Lambert AJ, Lobocka M, Nibert ML, Oksanen HM, Orton RJ, Robertson DL, Rubino L, Sabanadzovic S, Simmonds P, Smith DB, Suzuki N, Van Dooerslaer K, Vandamme AM, Varsani A, Zerbini FM (2021) Changes to virus taxonomy and to the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2021). Arch Virol 166:2633–2648

16. Zerbini FM, Siddell SG, Mushegian AR, Walker PJ, Lefkowitz EJ, Adriaenssens EM, Alfenas-Zerbini P, Dutilh BE, García ML, Junglen S, Krupovic M, Kuhn JH, Lambert AJ, Łobocka M, Oksanen HM, Robertson DL, Rubino L, Sabanadzovic S, Simmonds P, Suzuki N, Van Doorslaer K, Vandamme AM, Varsani A (2022) Differentiating between viruses and virus species by writing their names correctly. Arch Virol 167:1231–1234

17. Muhire BM, Varsani A, Martin DP (2014) SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. PLoS ONE 9:e108277

18. Zerbini FM, Siddell SG, Lefkowitz EJ, Mushegian AR, Adriaenssens EM, Alfenas-Zerbini P, Dempsey DM, Dutilh BE, García ML, Hendrickson RC, Junglen S, Krupovic M, Kuhn JH, Lambert AJ, Łobocka M, Oksanen HM, Robertson DL, Rubino L, Sabanadzovic S, Simmonds P, Smith DB, Suzuki N, Van Doorslaer K, Vandamme AM, Varsani A (2023) Changes to virus taxonomy and the ICTV Statutes ratified by the International Committee on Taxonomy of Viruses (2023). Arch Virol 168:175

19. Ilyina TV, Koonin EV (1992) Conserved sequence motifs in the initiator proteins for rolling circle DNA replication encoded by diverse replicons from eubacteria, eucaryotes and archaebacteria. Nucleic Acids Res 20:3279–3285

20. Chandler M, de la Cruz F, Dyda F, Hickman AB, Moncalian G, Ton-Hoang B (2013) Breaking and joining single-stranded DNA: the HUH endonuclease superfamily. Nat Rev Microbiol 11:525–538

21. Koonin EV, Ilyina TV (1993) Computer-assisted dissection of rolling circle DNA replication. Biosystems 30:241–268

22. Gorbalenya AE, Koonin EV (1993) Helicases: amino acid sequence comparisons and structure-function relationships. Curr Opin Struc Biol 3:419–429

23. Gorbalenya AE, Koonin EV, Wolf YI (1990) A new superfamily of putative NTP-binding domains encoded by genomes of small DNA and RNA viruses. FEBS Lett 262:145–148

24. Kazlauskas D, Dayaram A, Kraberger S, Goldstien S, Varsani A, Krupovic M (2017) Evolutionary history of ssDNA bacilladnaviruses features horizontal acquisition of the capsid gene from ssRNA nodaviruses. Virology 504:114–121

25. Crooks GE, Hon G, Chandonia JM, Brenner SE (2004) WebLogo: a sequence logo generator. Genome Res 14:1188–1190

26. 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

27. Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772

28. Capella-Gutierrez S, Silla-Martinez JM, Gabaldon T (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25:1972–1973

29. Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780

30. Stöver BC, Müller KF (2010) TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. BMC Bioinformatics 11:7

31. Darriba D, Taboada GL, Doallo R, Posada D (2011) ProtTest 3: fast selection of best-fit models of protein evolution. Bioinformatics 27:1164–1165

32. Wright EA, Heckel T, Groenendijk J, Davies JW, Boulton MI (1997) Splicing features in maize streak virus virion- and complementary-sense gene expression. Plant J 12:1285–1297

33. Todd D, Scott AN, Fringuelli E, Shivraprasad HL, Gavier-Widen D, Smyth JA (2007) Molecular characterization of novel circoviruses from finch and gull. Avian Pathol 36:75–81

34. Stewart ME, Perry R, Raidal SR (2006) Identification of a novel circovirus in Australian ravens (Corvus coronoides) with feather disease. Avian Pathol 35:86–92

35. Niagro FD, Forsthoefel AN, Lawther RP, Kamalanathan L, Ritchie BW, Latimer KS, Lukert PD (1998) Beak and feather disease virus and porcine circovirus genomes: intermediates between the geminiviruses and plant circoviruses. Arch Virol 143:1723–1744

36. Levy H, Fiddaman SR, Djurhuus A, Black CE, Kraberger S, Smith AL, Hart T, Varsani A (2020) Identification of Circovirus Genome in a Chinstrap Penguin (Pygoscelis antarcticus) and Adélie Penguin (Pygoscelis adeliae) on the Antarctic Peninsula. Viruses 12:858

37. Morandini V, Dugger KM, Ballard G, Elrod M, Schmidt A, Ruoppolo V, Lescroël A, Jongsomjit D, Massaro M, Pennycook J, Kooyman GL, Schmidlin K, Kraberger S, Ainley DG, Varsani A (2019) Identification of a Novel Adélie Penguin Circovirus at Cape Crozier (Ross Island, Antarctica). Viruses 11:1088

38. Kinsella CM, Bart A, Deijs M, Broekhuizen P, Kaczorowska J, Jebbink MF, van Gool T, Cotten M, van der Hoek L (2020) Entamoeba and Giardia parasites implicated as hosts of CRESS viruses. Nat Commun 11:4620

39. Krupovic M, Varsani A (2022) *Naryaviridae*, *Nenyaviridae*, and Vilyaviridae: three new families of single-stranded DNA viruses in the phylum *Cressdnaviricota*. Arch Virol 167:2907–2921

40. Cerna GM, Serieys LEK, Riley SPD, Richet C, Kraberger S, Varsani A (2023) A circovirus and cycloviruses identified in feces of bobcats (Lynx rufus) in California. Arch Virol 168:23

41. Custer JM, White R, Taylor H, Schmidlin K, Fontenele RS, Stainton D, Kraberger S, Briskie JV, Varsani A (2022) Diverse single-stranded DNA viruses identified in New Zealand (Aotearoa) South Island robin (Petroica australis) fecal samples. Virology 565:38–51

42. Fritz M, Reggiardo B, Filloux D, Claude L, Fernandez E, Mahé F, Kraberger S, Custer JM, Becquart P, Mebaley TN, Kombila LB, Lenguiya LH, Boundenga L, Mombo IM, Maganga GD, Niama FR, Koumba J-S, Ogliastro M, Yvon M, Martin DP, Blanc S, Varsani A, Leroy E, Roumagnac P (2023) African army ants at the forefront of virome surveillance in a remote tropical forest. Peer Community Journal 3:e24

43. Hess SC, Weiss KCB, Custer JM, Lewis JS, Kraberger S, Varsani A (2023) Identification of small circular DNA viruses in coyote fecal samples from Arizona (USA). Arch Virol 169:12

44. Lorincz M, Csagola A, Farkas SL, Szekely C, Tuboly T (2011) First detection and analysis of a fish circovirus. J Gen Virol 92:1817–1821

45. Lima FES, Cibulski SP, Bello AGD, Mayer FQ, Witt AA, Roehe PM, d'Azevedo PA (2015) A novel chiropteran circovirus genome recovered from a Brazilian insectivorous bat species. Genome Announc 3:01393–01415

46. Alex CE, Fahsbender E, Altan E, Bildfell R, Wolff P, Jin L, Black W, Jackson K, Woods L, Munk B, Tse T, Delwart E, Pesavento PA (2020) Viruses in unexplained encephalitis cases in American black bears (Ursus americanus). PLoS ONE 15:e0244056

47. Wu Z, Ren X, Yang L, Hu Y, Yang J, He G, Zhang J, Dong J, Sun L, Du J, Liu L, Xue Y, Wang J, Yang F, Zhang S, Jin Q (2012) Virome analysis for identification of novel mammalian viruses in bat species from Chinese provinces. J Virol 86:10999–11012

48. Todd D, Weston J, Ball NW, Borghmans BJ, Smyth JA, Gelmini L, Lavazza A (2001) Nucleotide sequence-based identification of a novel circovirus of canaries. Avian Pathol 30:321–325

49. Li L, McGraw S, Zhu K, Leutenegger CM, Marks SL, Kubiski S, Gaffney P, Dela Cruz FN Jr., Wang C, Delwart E, Pesavento PA (2013) Circovirus in tissues of dogs with vasculitis and hemorrhage. Emerg Infect Dis 19:534–541

50. Lorincz M, Dan A, Lang M, Csaba G, Toth AG, Szekely C, Csagola A, Tuboly T (2012) Novel circovirus in European catfish (*Silurus glanis*). Arch Virol 157:1173–1176

51. He B, Li Z, Yang F, Zheng J, Feng Y, Guo H, Li Y, Wang Y, Su N, Zhang F, Fan Q, Tu C (2013) Virome profiling of bats from Myanmar by metagenomic analysis of tissue samples reveals more novel Mammalian viruses. PLoS ONE 8:e61950

52. Wu Z, Yang L, Ren X, He G, Zhang J, Yang J, Qian Z, Dong J, Sun L, Zhu Y, Du J, Yang F, Zhang S, Jin Q (2016) Deciphering

the bat virome catalog to better understand the ecological diversity of bat viruses and the bat origin of emerging infectious diseases. ISME J 10:609–620

53. Nishizawa T, Sugimoto Y, Takeda T, Kodera Y, Hatano Y, Takahashi M, Okamoto H (2018) Identification and full-genome characterization of novel circoviruses in masked palm civets (Paguma larvata). Virus Res 258:50–54

54. Wu Z, Lu L, Du J, Yang L, Ren X, Liu B, Jiang J, Yang J, Dong J, Sun L, Zhu Y, Li Y, Zheng D, Zhang C, Su H, Zheng Y, Zhou H, Zhu G, Li H, Chmura A, Yang F, Daszak P, Wang J, Liu Q, Jin Q (2018) Comparative analysis of rodent and small mammal viromes to better understand the wildlife origin of emerging infectious diseases. Microbiome 6:178

55. Hattermann K, Schmitt C, Soike D, Mankertz A (2003) Cloning and sequencing of duck circovirus (DuCV). Arch Virol 148:2471–2480

56. Fisher M, Harrison TMR, Nebroski M, Kruczkiewicz P, Rothenburger JL, Ambagala A, Macbeth B, Lung O (2020) Discovery and comparative genomic analysis of elk circovirus (ElkCV), a novel circovirus species and the first reported from a cervid host. Sci Rep 10:19548

57. Li L, Kapoor A, Slikas B, Bamidele OS, Wang C, Shaukat S, Masroor MA, Wilson ML, Ndjango JB, Peeters M, Gross-Camp ND, Muller MN, Hahn BH, Wolfe ND, Triki H, Bartkus J, Zaidi SZ, Delwart E (2010) Multiple diverse circoviruses infect farm animals and are commonly found in human and chimpanzee feces. J Virol 84:1674–1682

58. Bandoo RA, Bautista J, Lund M, Newkirk E, Squires J, Varsani A, Kraberger S (2021) Identification of novel circovirus and anelloviruses from wolverines using a non-invasive faecal sampling approach. Infect Genet Evol 93:104914

59. Todd D, Weston JH, Soike D, Smyth JA (2001) Genome sequence determinations and analyses of novel circoviruses from goose and pigeon. Virology 286:354–362

60. Fehér E, Kaszab E, Bali K, Hoitsy M, Sós E, Bányai K (2022) Novel circoviruses from birds share common evolutionary roots with fish origin circoviruses. Life (Basel) 12:368

61. Lund MC, Larsen BB, Rowsey DM, Otto HW, Gryseels S, Kraberger S, Custer JM, Steger L, Yule KM, Harris RE, Worobey M, Van Doorslaer K, Upham NS, Varsani A (2023) Using archived and biocollection samples towards deciphering the DNA virus diversity associated with rodent species in the families cricetidae and heteromyidae. Virology 585:42–60

62. Zhu A, Jiang T, Hu T, Mi S, Zhao Z, Zhang F, Feng J, Fan Q, He B, Tu C (2018) Molecular characterization of a novel bat-associated circovirus with a poly-T tract in the 3' intergenic region. Virus Res 250:95–103

63. Hui A, Altan E, Slovis N, Fletcher C, Deng X, Delwart E (2021) Circovirus in blood of a febrile horse with hepatitis. Viruses 13:944

64. Gainor K, Becker A, Malik YS, Ghosh S (2021) Detection and complete genome analysis of circoviruses and cycloviruses in the small Indian Mongoose (Urva auropunctata): identification of novel species. Viruses 13:1700

65. Lian H, Liu Y, Li N, Wang Y, Zhang S, Hu R (2014) Novel circovirus from mink, China. Emerg Infect Dis 20:1548–1550

66. Payne N, Kraberger S, Fontenele RS, Schmidlin K, Bergeman MH, Cassaigne I, Culver M, Varsani A, Van Doorslaer K (2020) Novel circoviruses detected in Feces of Sonoran Felids. Viruses 12:1027

67. Sadeghi M, Altan E, Deng X, Barker CM, Fang Y, Coffey LL, Delwart E (2018) Virome of > 12 thousand Culex mosquitoes from throughout California. Virology 523:74–88

68. Khalifeh A, Custer JM, Kraberger S, Varsani A (2021) Novel viruses belonging to the family Circoviridae identified in wild American wigeon samples. Arch Virol 166:3437–3441

69. Wang B, Sun L-D, Liu H-H, Wang Z-D, Zhao Y-K, Wang W, Liu Q (2018) Molecular detection of novel circoviruses in ticks in northeastern China. Ticks Tick-borne Dis 9:836–839

70. Mankertz A, Hattermann K, Ehlers B, Soike D (2000) Cloning and sequencing of columbid circovirus (coCV), a new circovirus from pigeons. Arch Virol 145:2469–2479

71. Lecis R, Mucedda M, Pidinchedda E, Zobba R, Pittau M, Alberti A (2020) Genomic characterization of a novel bat-associated Circovirus detected in European Miniopterus schreibersii bats. Virus Genes 56:325–328

72. Hamel AL, Lin LL, Nayar GP (1998) Nucleotide sequence of porcine circovirus associated with postweaning multisystemic wasting syndrome in pigs. J Virol 72:5262–5267

73. Palinski R, Piñeyro P, Shang P, Yuan F, Guo R, Fang Y, Byers E, Hause BM (2017) A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure. J Virol 91:e01879-16

74. Zhang HH, Hu WQ, Li JY, Liu TN, Zhou JY, Opriessnig T, Xiao CT (2020) Novel circovirus species identified in farmed pigs designated as Porcine circovirus 4, Hunan province, China. Transbound Emerg Dis 67:1057–1061

75. Zhu A, Jiang T, Hu T, Mi S, Zhao Z, Zhang F, Feng J, Fan Q, He B, Tu C (2018) Molecular characterization of a novel bat-associated circovirus with a poly-T tract in the 3' intergenic region. Virus Res 250:95–103

76. Johne R, Fernández-de-Luco D, Höfle U, Müller H (2006) Genome of a novel circovirus of starlings, amplified by multiply primed rolling-circle amplification. J Gen Virol 87:1189–1195

77. Halami MY, Nieper H, Müller H, Johne R (2008) Detection of a novel circovirus in mute swans (Cygnus olor) by using nested broad-spectrum PCR. Virus Res 132:208–212

78. Patterson QM, Kraberger S, Martin DP, Shero MR, Beltran RS, Kirkham AL, Aleamotu'a M, Ainley DG, Kim S, Burns JM, Varsani A (2021) Circoviruses and cycloviruses identified in Weddell seal fecal samples from McMurdo Sound, Antarctica. Infect Genet Evol 95:105070

79. Bolatti EM, Viarengo G, Zorec TM, Cerri A, Montani ME, Hosnjak L, Casal PE, Bortolotto E, Di Domenica V, Chouhy D, Allasia MB, Barquez RM, Poljak M, Giri AA (2022) Viral metagenomic data analyses of five new world bat species from argentina: identification of 35 novel DNA viruses. Microorganisms 10:266

80. Landrau-Giovannetti N, Subramaniam K, Brown MA, Ng TFF, Rotstein DS, West K, Frasca S Jr, Waltzek TB (2020) Genomic characterization of a novel circovirus from a stranded Longman's beaked whale (Indopacetus pacificus). Virus Res 277:197826

81. Tokarz R, Lipkin WI (2021) Discovery and Surveillance of Tick-Borne Pathogens. J Med Entomol 58:1525–1535

82. Rinder M, Schmitz A, Peschel A, Korbel R (2015) Complete genome sequence of a novel circovirus from zebra finch. Genome Announc 3:e00560–15

83. Rosario K, Mettel KA, Benner BE, Johnson R, Scott C, Yusseff-Vanegas SZ, Baker CCM, Cassill DL, Storer C, Varsani A, Breitbart M (2018) Virus discovery in all three major lineages of terrestrial arthropods highlights the diversity of single-stranded DNA viruses associated with invertebrates. PeerJ 6:e5761

84. Li L, Shan T, Soji OB, Alam MM, Kunz TH, Zaidi SZ, Delwart E (2011) Possible cross-species transmission of circoviruses and cycloviruses among farm animals. J Gen Virol 92:768–772

85. Dayaram A, Potter KA, Moline AB, Rosenstein DD, Marinov M, Thomas JE, Breitbart M, Rosario K, Arguello-Astorga GR, Varsani A (2013) High global diversity of cycloviruses amongst dragonflies. J Gen Virol 94:1827–1840

86. Lima FE, Cibulski SP, Dos Santos HF, Teixeira TF, Varela AP, Roehe PM, Delwart E, Franco AC (2015) Genomic

characterization of novel circular ssDNA viruses from insectivorous bats in Southern Brazil. PLoS ONE 10:e0118070

87. Li L, Giannitti F, Low J, Keyes C, Ullmann LS, Deng X, Aleman M, Pesavento PA, Pusterla N, Delwart E (2015) Exploring the virome of diseased horses. J Gen Virol 96:2721–2733

88. Dunay E, Rukundo J, Atencia R, Cole MF, Cantwell A, Emery Thompson M, Rosati AG, Goldberg TL (2023) Viruses in saliva from sanctuary chimpanzees (Pan troglodytes) in Republic of Congo and Uganda. PLoS ONE 18:e0288007

89. Harding C, Larsen BB, Otto HW, Potticary AL, Kraberger S, Custer JM, Suazo C, Upham NS, Worobey M, Van Doorslaer K, Varsani A (2023) Diverse DNA virus genomes identified in fecal samples of Mexican free-tailed bats (Tadarida brasiliensis) captured in Chiricahua Mountains of southeast Arizona (USA). Virology 580:98–111

90. Zhang W, Li L, Deng X, Kapusinszky B, Pesavento PA, Delwart E (2014) Faecal virome of cats in an animal shelter. J Gen Virol 95:2553–2564

91. Phan TG, Luchsinger V, Avendano LF, Deng X, Delwart E (2014) Cyclovirus in nasopharyngeal aspirates of Chilean children with respiratory infections. J Gen Virol 95:922–927

92. Li L, Victoria JG, Wang C, Jones M, Fellers GM, Kunz TH, Delwart E (2010) Bat guano virome: predominance of dietary viruses from insects and plants plus novel mammalian viruses. J Virol 84:6955–6965

93. Ge X, Li J, Peng C, Wu L, Yang X, Wu Y, Zhang Y, Shi Z (2011) Genetic diversity of novel circular ssDNA viruses in bats in China. J Gen Virol 92:2646–2653

94. Fehér E, Kaszab E, Forró B, Bali K, Marton S, Lengyel G, Bányai K (2017) Genome sequence of a mallard duck origin cyclovirus, DuACyV-1. Arch Virol 162:3925–3929

95. Rosario K, Dayaram A, Marinov M, Ware J, Kraberger S, Stainton D, Breitbart M, Varsani A (2012) Diverse circular ssDNA viruses discovered in dragonflies (Odonata: Epiprocta). J Gen Virol 93:2668–2681

96. Harding C, Larsen BB, Gryseels S, Otto HW, Suazo C, Kraberger S, Upham NS, Worobey M, Van Doorslaer K, Varsani A (2022) Discovery of three cycloviruses in fecal samples from silver-haired bats (Lasionycteris noctivagans) in Arizona (USA). Arch Virol 167:2771–2775

97. Phan TG, Mori D, Deng X, Rajindrajith S, Ranawaka U, Fan Ng TF, Bucardo-Rivera F, Orlandi P, Ahmed K, Delwart E (2015) Small circular single stranded DNA viral genomes in unexplained cases of human encephalitis, diarrhea, and in untreated sewage. Virology 482:98–104

98. Smits SL, Zijlstra EE, van Hellemond JJ, Schapendonk CM, Bodewes R, Schurch AC, Haagmans BL, Osterhaus AD (2013) Novel cyclovirus in human cerebrospinal fluid, Malawi, 2010–2011. Emerg Infect Dis 19:1511–1513

99. 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

100. Tan le V, van Doorn HR, Nghia HD, Chau TT, Tu le TP, de Vries M, Canuti M, Deijs M, Jebbink MF, Baker S, Bryant JE, Tham NT, NT BK, Boni MF, Loi TQ, Phuong le T, Verhoeven JT, Crusat M, Jeeninga RE, Schultsz C, Chau NV, Hien TT, van der Hoek L, Farrar J, de Jong MD (2013) Identification of a new cyclovirus in cerebrospinal fluid of patients with acute central nervous system infections. mBio 4:e00231-13

101. Male MF, Kraberger S, Stainton D, Kami V, Varsani A (2016) Cycloviruses, gemycircularviruses and other novel replication-associated protein encoding circular viruses in Pacific flying fox (Pteropus tonganus) faeces. Infect Genet Evol 39:279–292

102. Sato G, Kawashima T, Kiuchi M, Tohya Y (2015) Novel cyclovirus detected in the intestinal contents of Taiwan squirrels (Callosciurus erythraeus thaiwanensis). Virus Genes 51:148–151

103. Padilla-Rodriguez M, Rosario K, Breitbart M (2013) Novel cyclovirus discovered in the Florida woods cockroach Eurycotis floridana (Walker). Arch Virol 158:1389–1392

104. Fontenele RS, Lacorte C, Lamas NS, Schmidlin K, Varsani A, Ribeiro SG (2019) Single stranded DNA viruses associated with capybara faeces sampled in Brazil. Viruses 11:710