Evaluation of bat adenoviruses suggests co-evolution and host roosting behaviour as drivers for diversity

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Abstract

Adenoviruses (AdVs) are diverse pathogens of humans and animals, with several dozen bat AdVs already identified. Considering that over 100 human AdVs are known, and the huge diversity of bat species, many bat AdVs likely remain undiscovered. To learn more about AdV prevalence, diversity and evolution, we sampled and tested bats in Cameroon using several PCR assays for viral and host DNA. AdV DNA was detected in 14% of the 671 sampled animals belonging to 37 different bat species. There was a correlation between species roosting in larger groups and AdV DNA detection. The detected AdV DNA belonged to between 28 and 44 different, mostly previously unknown, mastadenovirus species. The novel isolates are phylogenetically diverse and while some cluster with known viruses, others appear to form divergent new clusters. The phylogenetic tree of novel and previously known bat AdVs does not mirror that of the various host species, but does contain structures consistent with a degree of virus–host co-evolution. Given that closely related isolates were found in different host species, it seems likely that at least some bat AdVs have jumped species barriers, probably in the more recent past; however, the tree is also consistent with such events having taken place throughout bat AdV evolution. AdV diversity was highest in bat species roosting in large groups. The study significantly increased the diversity of AdVs known to be harboured by bats, and suggests that host behaviours, such as roosting size, may be what limits some AdVs to one species rather than an inability of AdVs to infect other related hosts.

DATA SUMMARY

Ten supplementary data files (Files S1–S10) for this study can be found on Figshare at https://figshare.com/s/31d9a89820f7bcea32c2. Novel viral sequences are listed in File S1 and have been deposited in GenBank with accession numbers MN136540–MN136633. Previously published sequences were downloaded from GenBank, their accession numbers are listed in File S2.

INTRODUCTION

Adenoviruses (AdVs) have been intensely studied over several decades, initially due primarily to their roles as human pathogens, but more recently with an additional focus on their prospective use as tools in gene therapy. Research originally focused on a handful of serotypes, but advances in sequencing technology have led to the discovery of close to 100 different human AdV types (HAdVs) [1–4] [http://hadvwg.gmu.edu]. Humans, however, are not the only species infected by AdVs. Members of the diverse family adenoviridae have been shown to infect a wide variety of vertebrate species, including rodents and bats. AdVs are generally considered host-specific viruses, with a few exceptions including CAdV-1 (canine), which infects a broad set of carnivores, and some SAdVs (simian), where transmission between primates and humans has been documented or suspected [1, 5–10].

Taking the general host specificity of AdVs into account, each host species should have its own set of AdVs, though for most species other than humans we only know of very few, one or no AdVs per host. Considering the high diversity of HAdVs, it seems likely that there may be many AdV types in each of many host species which have remained undetected so far. Identifying and studying these viruses can provide insights into what drives AdV evolution, mechanisms of pathogenicity, and general virus and host biology. The most promising hosts in which to look for AdVs in terms of characterizing evolution are certainly rodents and bats, as these two orders alone account for over 70% of all living mammal species, and recent studies have indeed unveiled many previously unknown AdVs in these hosts [2, 11–16].

Bats in particular have proven to be a rich source of viral discoveries in recent years and have been the subject of many studies evaluating the risks of zoonotic viral transmission from bats to humans or to other hosts. Viruses of significant impact on humans such as Ebola, corona and rabies viruses in bats have been extensively studied but AdVs, while less extensively studied, have also been detected in various species of bats from different continents with prevalences of up to almost 15% [14–23]. Previous studies mostly support a high diversity and the general pattern of virus–host co-evolution in AdVs; however, there is evidence that the latter principle may not be as strict as previously thought [23, 24].

The present study was part of the global United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT project, aiming at the identification of viral risks at the human–animal interface. The goal of this specific study was to test bats in Cameroon for evidence of current AdV infection to evaluate (i) the AdV (DNA) prevalence in the tested population, (ii) the diversity of AdVs among the bats, (iii) correlations between host species and detected viruses, and (iv) to determine what these can tell us about AdV evolution.

METHODS

Sampling

Samples from bats were collected between January 2009 and April 2013 in multiple locations in Cameroon (CMR) (Fig. 1, File S3). Bats were obtained from hunters who were willing to let us sample the individuals they captured. Opportunistic samples were taken and hunted bats returned to the hunter after specimen collection. Two specimens of about 200 mg (pea-size) samples from liver and spleen were collected. For each tissue, one specimen was placed in an empty 1.5 ml cryovial and the other in 1.5 ml cryotube containing 500 μl RNA*later* (Qiagen). All specimens were frozen immediately in liquid nitrogen in the field and later stored at −80 °C at the laboratory until further processing was carried out.

PCR and sequence analysis

DNA was extracted from the specimens using a Qiagen AllPrep DNA/RNA kit, according to the manufacturer's

Impact Statement

Adenoviruses (AdVs) play a role as human pathogens, but they are also used or candidates for applications in vaccine delivery and gene therapy. We are, however, still learning about the evolutionary mechanisms of this viral family, and bats as one of the most diverse mammalian orders are ideal to study viral diversity and evolution in the field. The detected evidence of many novel bat AdV types helps in the understanding of this, and our findings support a high degree of complexity in AdV evolution. The findings also suggest host behaviour as an important driver in spread and potentially in diversification. This principle may also be relevant beyond bat AdVs.

instructions. DNA was stored at −20 °C until analysis. A nested PCR was used to screen for AdV DNA in pooled liver/spleen samples separate for each bat. The selected PCR targets the conserved DNA polymerase gene and amplifies a product of approximately 270–276 nt flanked by the primer binding sites. The primers for the first round (polFouter, 5′-TIM GNG GIG GIM GNT GYT AYC C-3′, and polRouter, 5′-GTD GCR AAI SHI CCR TAB ARI GMR TT-3′) as well as for the second round (polFinner, 5′-GTI TWY GAY ATH TGY GGH ATG TAY GC-3′, and polRinner, 5′-CCA ICC BCD RTT RTG IAR IGT RA-3′) of the PCR are degenerated to enable the detection of a variety of AdVs [25].

To confirm the correct field-identification of bats, DNA samples from representatives of different field-identified species were tested with a cytochrome b (CytB) PCR assay and the products were sequenced. The primers CytB_F (5′-GAG GMC AAA TAT CAT TCT GAG G-3′) and CytB_R (5′-TAG GGC VAG GAC TCC TCC TAG T-3′) were used to amplify a primer-flanked 435 nt fragment of the highly conserved mitochondrial gene [26].

For visualization, all PCR products were loaded into and run in a 1.5% agarose gel, and products corresponding to the expected size were excised. DNA was extracted using either the Qiagen QIAquick gel extraction kit or the MP Biomedicals GeneClean kit and were sent for commercial Sanger sequencing at GATC Biotech. Sequencing results were assessed and processed using Geneious 7.1, and compared to the GenBank database (BLAST N).

Two phylogenetic trees were reconstructed for viral sequences, one based on the PCR target region in the polymerase gene, and one based on available sequences of the full polymerase gene. For the host species, full or close to full (>1029 nt) CytB sequences were used for phylogenetic analysis. Published bat AdV DNA sequences covering the PCR target region (*n*=178) were included in the PCR-based tree, provided they did not match other published sequences by more than 90%, in which case only one (the first in the preliminary multiple sequence alignment) of the putative species was included (File S2). Novel isolates with more than 90% nucleotide identities to
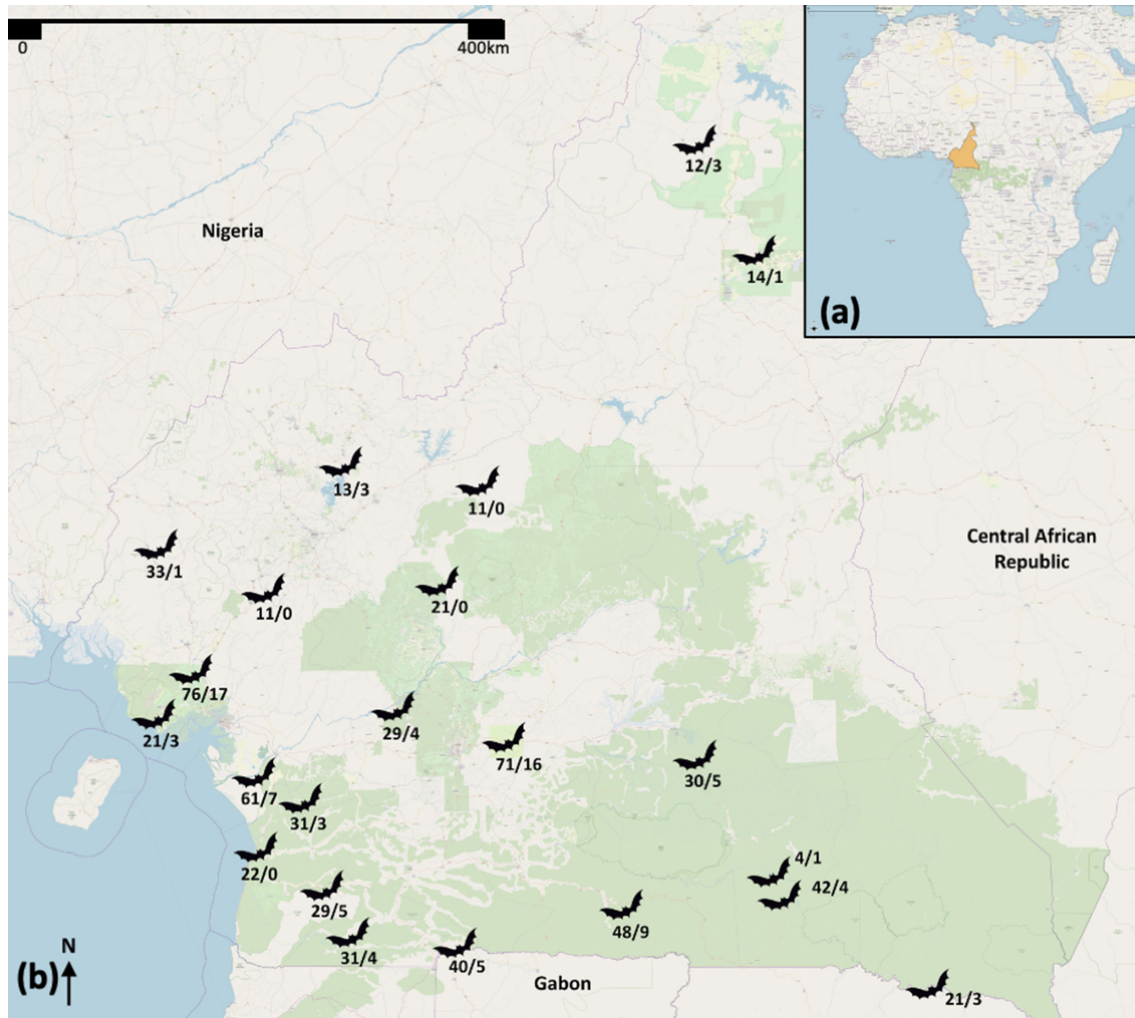
Fig. 1. Sampling locations: map of the sampling sites highlighting Cameroon's location in Africa (a) and indicating the total number of animals sampled and the number of AdV-positive animals from that site (total number/number positive) (b); see File S3 for more details.

other new isolates were also omitted during reconstruction, but are marked in the tree next to included ones. The same 90% nucleotide identity cut-off was used as an inclusion criterion for the full polymerase gene tree. The host CytB sequences selected represent AdV hosts reported either previously or in this study.

For the purpose of phylogenetic analysis, multiple sequence alignments were made in Geneious (version 11.1.3, MUSCLE alignment) (Files S4–S6). Bayesian phylogenies were inferred using MrBayes 3.2 [27]: datatype=DNA, Nucmodel=4by4, Nst=1, Coavion=no, no. states=4, rates=equal, two runs; four chains of 50000000 generations for virus PCR target regions, 10000000 generations for host gene sequences and 1000000 for virus full polymerase gene sequences. *Fowl aviadenovirus 1* and *2*, as members of the genus *Aviadenovirus*, served as the outgroup to root the PCR trees, *Gallus gallus* and *Anas platyrhynchos* were used as the outgroup for CytB trees, and *Murid adenovirus 1* and *3* as the outgroup for virus full polymerase trees. Trees were sampled after every 1000 steps during the process to monitor phylogenetic convergence. The mean standard deviation of split frequencies was below 0.0184 for the AdV PCR target region analysis, below 0.0081 for the CytB analysis and 0 for the virus full polymerase analysis. The first 10% of the trees were discarded and the remaining ones combined using TreeAnnotator (versions 1.10.4 and 2.5.1; http://beast.bio.ed.ac.uk) and displayed with FigTree (1.4.4; http://tree.bio.ed.ac.uk/). Sequences obtained from the CytB PCR were 'blasted' against the GenBank database and were considered to match a species if identities were at 98% or higher.

Statistical analysis

Ecological data collected for each animal was analysed with respect to a correlation with the frequency of virus detection. We assessed the effect of sex (male vs female), season the sample was taken (rainy – May to September, dry – October to April) and colony behaviour on the likelihood of AdV DNA being detected in an individual. Colony behaviour

was coded as an ordinal variable, specifically categorizing species as solitary (code=1), mixed solitary and small colonies (code=2), small colonies (code=3), mixed small and large colonies (code=4), and bats that exhibit highly colony-based/gregarious behaviour based on published references and the IUCN (International Union for Conservation of Nature) Red List (Table 1) [28–30]. The likelihood of detecting AdV DNA in an individual animal based on these variables was assessed by fitting a multivariate general linear binomial model with a logit link (R v. 3.4.0). There were insufficient numbers of individuals in many species to consider species as a predictor, and so inferences are limited to the taxa 'bat'. We did, however, have sufficient samples of bats to compare the likelihood of detecting AdV DNA from the families *Hipposideridae* and *Pteropodidae* using a univariate analysis (Chi-square contingency table). There were insufficient numbers of bats from other families (i.e. *n* <35 for the remaining six families represented) to include family as a covariate in the model.

For cospeciation analysis, the Jane software tool (version 4) with default settings for generations and population size was used with the host and virus trees (Figs 2 and 3) [31]. In order to run the software virus sequences without the corresponding host, CytB was excluded as well as bat AdV species 15, 18 and 35. Default costs for events were used (cospeciation 0, duplications 1, duplications and host switches 2, losses 1, failure to diverge 1) and more extreme costs (10) for each category explored.

Correlations between the AdV phylogeny of the PCR-based tree and the geographical location of sample collection were evaluated using the BaTS software package [32]. Locations ('states') were either coded as the country or as the continent from which a sequence originated.

RESULTS

Roosting behaviour correlates with AdV prevalence

During the course of this study, 671 bats from 37 different species were sampled in various regions and habitats in Cameroon (Fig. 1, File S3). The most commonly sampled species were *Epomops franqueti* (124, 18.5%), *Hipposideros ruber* (108, 16.1%) and *Megaloglossus woermanni* (108, 16.1%), while other species were represented by fewer or only a single individual (Table 1). AdV DNA was detected in 94 (14%) of the bats, with detection rates ranging from 0 to 100% for various species. We were more likely to detect AdV DNA in samples from bats from the family *Hipposideridae* than the family *Pteropodidae* (Chi-square=14.34, df=1, *P* <0.001; *Hipposideridae* 21.7% positive, *n*=161; *Pteropodidae* 9.5% positive, *n*=411). We did not detect an effect of season (12.6% positive in the dry season vs 16.7% in the rainy season; β=0.34, SE=0.24, *P*=0.15, *n*=671) or sex (12.8% positive among females vs 15.6% positive among males; β=0.27, SE=0.23, *P*=0.24, *n*=666; 5 individuals with undetermined sex were excluded) (File S7). We did detect a correlation between degree of colony behaviour and proportion of individuals testing positive for AdV (β=0.28, SE=0.09, *P*=0.00145, *n*=654;

Table 1. Sampled bat species

Species	No. AdV positive/total no. sampled (% AdV positives)	Roosting behaviour
Pteropodidae		
Casinycteris argynnis	0/2 (0%)	Solitary
Eidolon helvum	1/1 (100%)	Large colonies
Epomophorus gambianus	0/9 (0%)	Large colonies
Epomops franqueti	5/124 (4%)	Small colonies
Hypsignathus monstrosus	1/4 (25%)	Small colonies
Lissonycteris angolensis	2/19 (10.5%)	Small colonies
Megaloglossus woermanni	14/108 (13%)	Solitary
Micropteropus pusillus	1/68 (1.5%)	Solitary
Myonycteris torquata	0/25 (0%)	Small colonies/solitary
Myonycteris sp.	2/2 (100%)	Unknown
Nanonycteris veldkampii	0/3 (0%)	Unknown
Rousettus aegyptiacus	15/43 (34.9%)	Large colonies
Scotonycteris zenkeri	0/5 (0%)	Small colonies
Hipposideridae		
Hipposideros beatus	0/2 (0%)	Small colonies/solitary
Hipposideros cyclops	15/20 (75%)	Small colonies
Hipposideros fuliginosus	0/3 (0%)	Small colonies
Hipposideros gigas	3/28 (10.7%)	Large colonies
Hipposideros ruber	17/108 (15.7%)	Large colonies/small colonies
Rhinolophidae		
Rhinolophus alcyone	4/5 (80%)	Small colonies/solitary
Rhinolophus fumigatus	1/1 (100%)	Large colonies
Rhinolophus landeri	0/8 (0%)	Small colonies
Rhinopomatidae		
Rhinopoma microphyllum	0/1 (0%)	Large colonies/small colonies
Molossidae		
Chaerephon pumilus	2/3 (66.7%)	Large colonies
Mops condylurus	3/25 (12%)	Large colonies/small colonies
Mops demonstrator	0/6 (0%)	Small colonies
Vespertilionidae		
Eptesicus capensis	0/2 (0%)	Small colonies

Table 1. Continued

Species	No. AdV positive/total no. sampled (% AdV positives)	Roosting behaviour
Neoromicia tenuipinnis	5/11 (45.5%)	Small colonies
Kerivoula cuprosa	0/4 (0%)	Unknown
Kerivoula sp.	0/1 (0%)	Unknown
Pipistrellus musciculus	1/4 (25%)	Unknown
Pipistrellus nanulus	0/2 (0%)	Small colonies/solitary
Miniopteridae		
Miniopterus inflatus	0/1 (0%)	Large colonies/small colonies
Miniopterus schreibersii	0/1 (0%)	Large colonies
Nycteridae		
Nycteris grandis	1/16 (6.3%)	Small colonies
Nycteris hispida	1/4 (25%)	Small colonies
Nycteris major	0/1 (0%)	Solitary
Nycteris thebaica	0/1 (0%)	Large colonies/small colonies
Total	94/671 (14%)	

17 animals from species with unknown roosting behaviour were excluded) (Fig. 4).

Detected AdV DNA belongs to different AdV species

The majority of the sequences obtained from the 94 AdV DNA positive samples did not resemble previously reported sequences. Only 22 of the isolates had GenBank matches with nucleotide identities of 85% or more, and only 2 of these (CMR-5952 and CMR-5697) were identical with known bat AdV sequences (Table 2). With a cut-off of 5–15% differences in the amino acid sequence, the 94 virus isolates could be classified into 28 to 44 different mastadenovirus species [2]. Upon phylogenetic analysis, novel isolates were found in all major branches of the tree alongside other AdV isolates from a diverse set of bat host species sampled in different locations in Africa, Asia and Europe, while some of the novel isolates formed exclusive clusters (Fig. 2). Phylogeny-trait association tests based on monophyletic clade (MC) size statistic of virus-location phylogenetic substructure do not reject the null hypothesis of no association between virus and location, as MC for all states (countries and continents) is >0.01.

Highly social bats seem to be infected by more AdV species

The proportion of samples with a unique AdV species (number of different AdV species detected/number of samples collected) differed depending on the behaviour category. Bats roosting in large colonies carried a significantly more diverse set of AdVs compared to all other categories combined (Chi-square=13.32, df=1, $P < 0.001$; 'large colonies' 15.1%, $n=86$; 'all other' 4.9%, $n=571$) or individually, while in solitary bats the proportion was the lowest (Chi-square=5, df=1, $P < 0.026$; 'solitary' 2.8%, $n=178$; 'all other' 7.5%, $n=478$).

In the 7 bat species that were roosting in large colonies and were sampled, a total of 13 distinct AdVs were detected (ratio of 1.9 AdVs per species), while in the 26 species in all the other categories combined, 28 distinct AdVs were detected (ratio of 1.1 AdVs per species). A similar trend for more AdV species among large colony roosting bats can be seen in the GenBank data set, with 11 AdV species deriving from 5 bat species (ratio of 2.2 AdVs per species) compared to 68 AdVs derived from 44 bat species across all other categories (ratio of 1.5 AdVs per species) (Files S2 and S8).

Virus and host phylogenies differ

A CytB-based phylogenetic analysis of all bat species from which AdV DNA was isolated and reported produced a tree consistent with current bat taxonomy (Fig. 3). The host and the PCR-fragment-based virus trees do not mirror each other, but branches in the virus tree are largely bat *suborder* specific and contain either viruses of *Yinpterochiroptera* or *Yangochiroptera* hosts. A virus tree calculated based on full genome sequences does not provide the same level of resolution due to the limited number of available sequences, but with high bootstrap supports indicates that the split of *Yinpterochiroptera* and *Yangochiroptera* bats is not well reflected in the infecting AdV's phylogeny (File S9). Most of the larger clusters in the PCR-fragment-based virus tree represent only one family of bats, but some families, such as *Vespertilionidae* and *Pteropodidae*, are represented with more than one of these exclusive clusters (Fig. 2). A consistent clustering down to the host genus level is not visible, but 75% (15/20) of the virus species detected in more than one animal were only found in one species (Table 2). Cospeciation analysis with Jane indicates diverse modes as drivers for the calculated phylogeny with cospeciation (9), duplication of parasites (27), duplication and host switch (52), loss of parasite (105), failure to diverge (18), at a total cost of 254.

DISCUSSION

We detected AdV DNA in 14% of the tested bats, which is close to the upper end of what had been reported previously and, thus, very similar to what Vidovsky *et al.* found in bats in Europe [14]. However, such an overall prevalence has to be considered with caution, as many ecological factors may have an influence on this. Like Vidovsky *et al.*, we found a large variation in prevalence between the bat species (0–100%), and the species composition of tested bat populations is probably one of the main factors contributing to the prevalence differences among studies [14–23]. The 37 bat species we sampled were not equally represented, with over a hundred individuals tested from each of three species and only a single or few individuals of some other species (Table 1). Therefore, the prevalences we observed in some species may not necessarily be representative. However, the 671 bats tested do
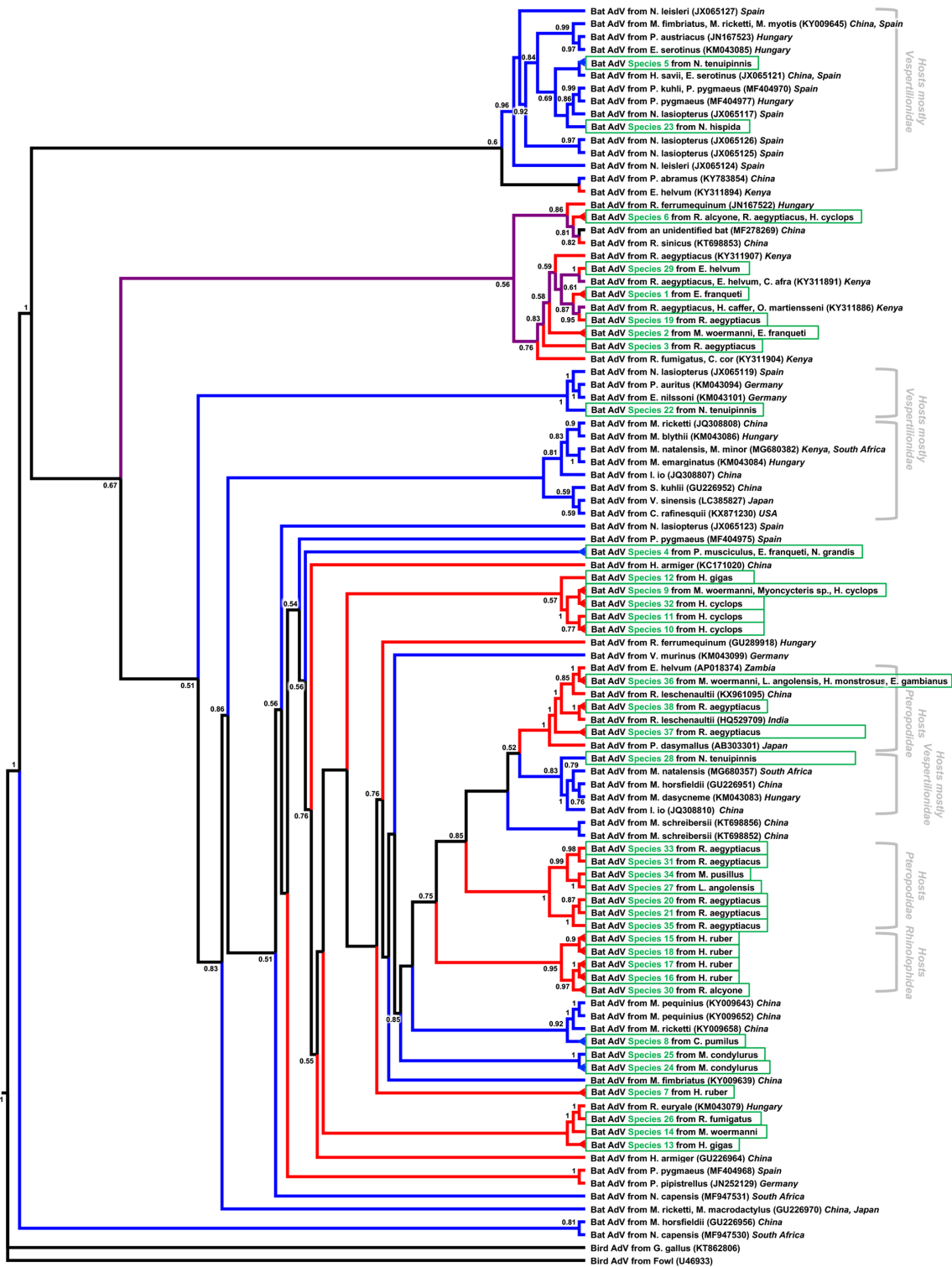
Fig. 2. Virus phylogeny: phylogenetic tree of bat AdVs based on the PCR target region (270–276 nt) of the PCR described by Wellehan *et al.* [25]. Previously published sequences are indicated by GenBank accession number, host species and sampling location, novel isolates (in green boxes) by species numbers and the hosts. Bootstrap support is shown on nodes if higher than 0.5. The branches of AdVs from *Yinpterochiroptera* bats are coloured in red, branches of AdVs from *Yangochiroptera* bats in blue, with purple indicating branches with viral isolates that were found in both. Branches with funnel like ends indicate that one sequence is representing several new isolates.
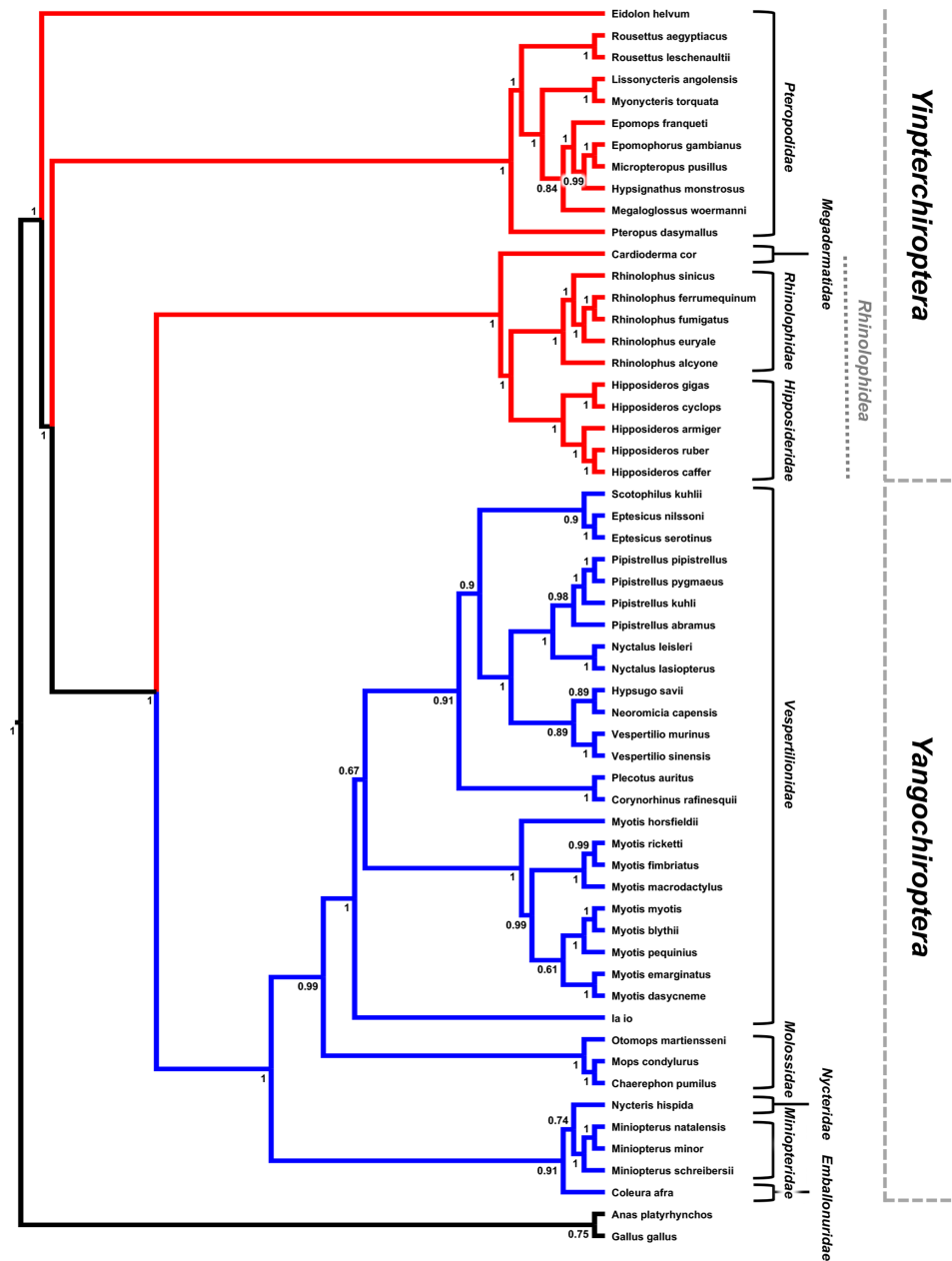
Fig. 3. Phylogenetic tree of bats: phylogenetic tree of bat species based on the full or near full cytochrome B gene. Bootstrap support is shown on knots. The branches of *Yinpterochiroptera* bats are coloured in red, branches of *Yangochiroptera* bats in blue. The sequences included are: AB355764 (*Eidolon helvum*), AB085740 (*Rousettus aegyptiacus*), FJ549336 (*Rousettus leschenaultii*), JN398205 (*Lissonycteris angolensis*), JF728759 (*Myonycteris torquata*), JF728758 (*Epomops franqueti*), KT875803 (*Epomophorus gambianus*), JN398208 (*Micropteropus pusillus*), KX823086 (*Hypsignathus monstrosus*), DQ445710 (*Megaloglossus woermanni*), AB042770 (*Pteropus dasymallus*), MH686226 (*Cardioderma cor*), EU434941 (*Rhinolophus sinicus*), DQ351848 (*Rhinolophus ferrumequinum*), FJ457614 (*Rhinolophus fumigatus*), EU436671 (*Rhinolophus euryale*), FJ457613 (*Rhinolophus alcyone*), EU934469 (*Hipposideros gigas*), EU934466 (*Hipposideros cyclops*), JX849190 (*Hipposideros armiger*), FJ347988 (*Hipposideros ruber*), FJ347980 (*Hipposideros caffer*), EU750931 (*Scotophilus kuhlii*), GQ272582 (*Eptesicus nilssoni*), EU751000 (*Eptesicus serotinus*), KF874515 (*Pipistrellus pipistrellus*), AJ504442 (*Pipistrellus pygmaeus*), KU058655 (*Pipistrellus kuhli*), CQ332430 (*Pipistrellus abramus*), JX570901 (*Nyctalus leisleri*), JX570900 (*Nyctalus lasiopterus*), AJ504450 (*Hypsugo savii*), KJ756000 (*Neoromicia capensis*), LC052293 (*Vespertilio murinus*), AB287362 (*Vespertilio sinensis*), EF570882 (*Plecotus auritus*), AY776085 (*Corynorhinus rafinesquii*), KP187907 (*Myotis horsfieldii*), KX467599 (*Myotis ricketti*), EF555226 (*Myotis fimbriatus*), AB085736 (*Myotis macrodactylus*), GU817388 (*Myotis myotis*), KX467606 (*Myotis blythii*), KX467608 (*Myotis pequinius*), AF376849 (*Myotis emarginatus*), AF376846 (*Myotis dasycneme*), MF615184 (*Ia io*), AY591536 (*Otomops martiensseni*), MK330941 (*Mops condylurus*), GQ489157 (*Chaerephon pumilus*), HQ693723 (*Nycteris hispida*), KX548053 (*Miniopterus natalensis*), FJ232806 (*Miniopterus minor*), AY208140 (*Miniopterus schreibersii*), JQ956449 (*Coleura afra*), EU755252 (*Anas platyrhynchos*) and AB04486 (*Gallus gallus*).
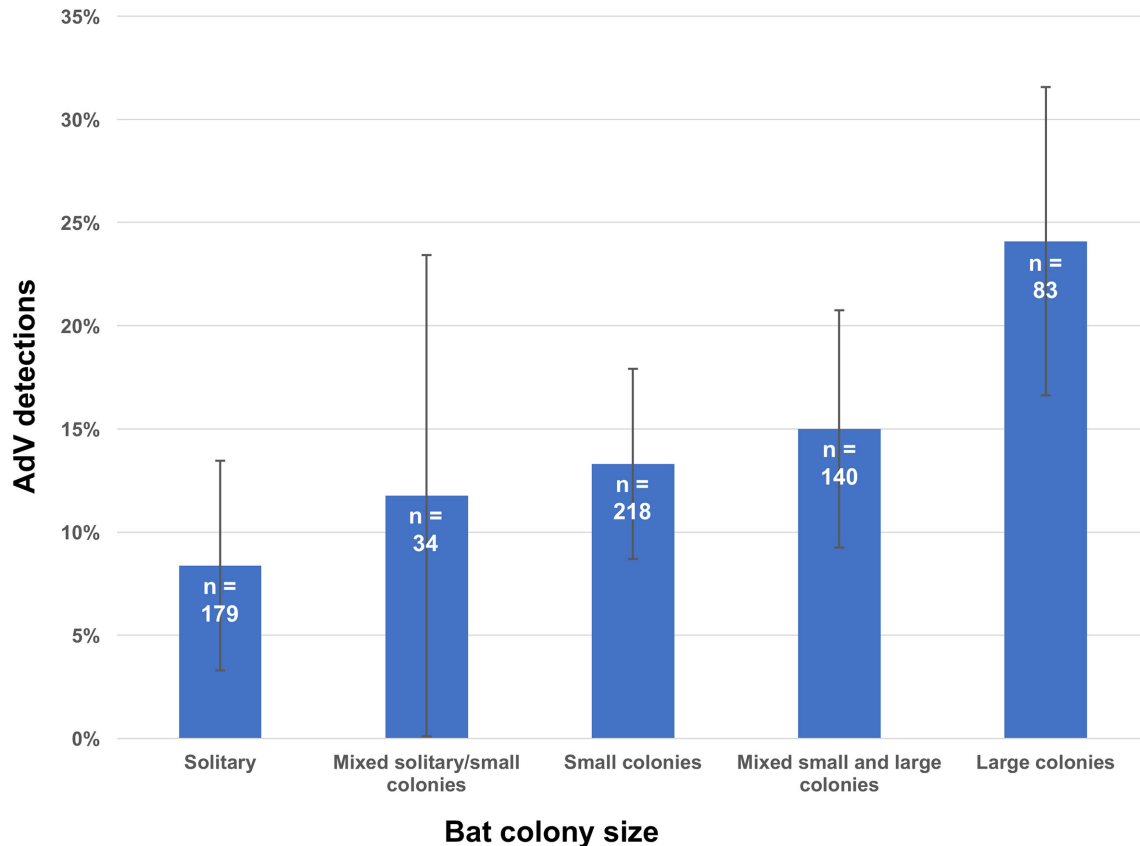
Fig. 4. Roosting behaviour correlates with AdV prevalence: proportion of virus detections in animals in relation to roosting behaviour. Total animals sampled per category and margin of error for 95% confidence intervals are shown.

represent a substantial range of species with different reported roosting behaviours, and the data suggests a correlation between bats dwelling in large caves or in big groups and bats having a higher prevalence of AdV infections (Fig. 4). This observation also aligns with what is known generally about AdV transmission, which has been shown to be more likely to be increased under crowded conditions, an example being HAdV outbreaks in army camps [1].

The diversity of the detected AdVs was high since, according to the International Committee on Taxonomy of Viruses (ICTV) criteria, 28–44 of the 94 detected sequences might represent unique AdV species, depending on where the line is drawn [2]. Based on the comparison of the amino acid sequences of the full gene and the PCR target region of 16 common mastadenoviruses, we conclude that the amplified region is generally more conserved than the whole gene; hence, the number of viral species may be closer to the higher end; for the tree we were assuming 38. Sequencing of the whole gene or full genomes would have allowed for a clearer classification; however, since for most other bat AdVs only the PCR region has been sequenced, it would not have had much influence on the analysis. Considering that the assumed 28–44 AdV species were detected in only 17 different bat species, we are looking at a ratio of between 1.6 and 2.6 AdV species per

host species. This is higher than what has been observed, for example, in rodents, and similar to what was found in bats elsewhere [14–16]. This supports the hypothesis that the diverse order Chiroptera might still harbour hundreds of yet unknown AdVs [14].

Virus–host co-evolution and species specificity have generally been regarded as the driving factors for AdV evolution and diversity, despite the relative host ambiguity of CAdV-1 [5, 7, 24]. However, recent findings, such as evidence for human–primate zoonotic events and results from surveys in rodents and bats, indicate that matters may be more complex [1, 6, 8–12, 14–16, 23]. The results of the Jane analysis, with relatively few (9) predicted co-speciation events and an overall high total cost of 254 (the higher the influence of co-speciation the closer it would be to 0), also point towards a complex evolution of bat AdVs that involves, but not primarily depends on, co-evolution. As a consequence of the majority of sequences obtained by us and others being relatively short, bootstrap support for many nodes in the phylogenetic tree is not optimal, so that the location of various nodes and branches has to be viewed with some caution (Fig. 2). Our analysis of a large number of bat AdV sequences, nevertheless, suggests that species specificity and co-evolution alone may not be sufficient to explain the observed diversity. The

Table 2. List of detected viral sequences

Species	Isolate/GenBank acc. no.	Host family and species	BLAST N (29/05/18)	Location*
1	CMR-5440, MN136559	*Pteropodidae, Epomops franqueti*	80% unidentified adenovirus isolate PgAdV-1 (KC692417)	ES
	CMR-5586, MN136577	*Pteropodidae, Epomops franqueti*	80% unidentified adenovirus isolate PgAdV-1 (KC692417)	SU
	CMR-5628, MN136584	*Pteropodidae, Epomops franqueti*	81% unidentified adenovirus isolate PgAdV-1 (KC692417)	SU
2	CMR-5524, MN136570	*Pteropodidae, Megaloglossus woermanni*	77% unidentified adenovirus isolate PgAdV-1 (KC692417)	ES
	CMR-5529, MN136573	*Pteropodidae, Megaloglossus woermanni*	76% unidentified adenovirus isolate PgAdV-1 (KC692417)	ES
	CMR-5531, MN136574	*Pteropodidae, Epomops franqueti*	77% unidentified adenovirus isolate PgAdV-1 (KC692417)	ES
	CMR-5646, MN136586	*Pteropodidae, Megaloglossus woermanni*	77% unidentified adenovirus isolate PgAdV-1 (KC692417)	SU
3	CMR-5587, MN136578	*Pteropodidae, Rousettus aegyptiacus*	99% unidentified adenovirus isolate CS/13HN77 (KT369241)	SU
4	CMR-5459, MN136560	*Vespertilionidae, Pipistrellus musciculus*	77% mastadenovirus sp. strain BatAdVNeoV17-2 (MF593274)	SU
	CMR-5470, MN136561	*Vespertilionidae, Epomops franqueti*	76% vespertilionid adenovirus 4 strain 492/08 (KM043107)	SU
	CMR-5481, MN136563	*Nycteridae, Nycteris grandis*	77% mastadenovirus sp. strain BatAdVNeoV17-2 (MF593274)	SU
5	CMR-5238, MN136544	*Vespertilionidae, Neoromicia tenuipinnis*	82% mastadenovirus sp. strain BtAdVNeoC52222 (MF593281)	LI
	CMR-5241, MN136545	*Vespertilionidae, Neoromicia tenuipinnis*	81% mastadenovirus sp. strain BtAdVNeoC52222 (MF593281)	LI
	CMR-5242, MN136546	*Vespertilionidae, Neoromicia tenuipinnis*	82% mastadenovirus sp. strain BtAdVNeoC52222 (MF593281)	LI
6	CMR-5263, MN136547	*Rhinolophidae, Rhinolophus alcyone*	74% bat mastadenovirus WIV10 (KT698854)	LI
	CMR-5679, MN136588	*Rhinolophidae, Rhinolophus alcyone*	75% bat mastadenovirus WIV11 (KT698855)	SW
	CMR-5984, MN136626	*Pteropodidae, Rousettus aegyptiacus*	77% bat mastadenovirus WIV10 (KT698854)	SW
	CMR-6223, MN136629	*Hipposideridae, Hipposideros cyclops*	75% bat mastadenovirus WIV11 (KT698855)	CE
7	CMR-5201, MN136540	*Hipposideridae, Hipposideros ruber*	71% unidentified adenovirus isolate Aau12186 (MF175106)	ES
	CMR-5713, MN136598	*Hipposideridae, Hipposideros ruber*	72% bat adenovirus isolate 1391-YN-Ha (GU226964)	ES
	CMR-5716, MN136600	*Hipposideridae, Hipposideros ruber*	71% canine mastadenovirus A isolate CAdV-1 ITL2015 (KX545420)	ES
8	CMR-5232, MN136543	*Molossidae, Chaerephon pumilus*	73% bat adenovirus strain C018/China/2015 (KY009658)	LI
	CMR-5929, MN136618	*Molossidae, Chaerephon pumilus*	74% bat adenovirus strain C018/China/2015 (KY009658)	N

Continued

Table 2. Continued

Species	Isolate/GenBank acc. no.	Host family and species	BLAST N (29/05/18)	Location*
9	CMR-5379, MN136549	*Hipposideridae, Hipposideros cyclops*	74% unidentified adenovirus isolate Aau12186 (MF175106)	ES
	CMR-5381, MN136550	*Hipposideridae, Hipposideros cyclops*	73% unidentified adenovirus isolate Aau12186 (MF175106)	ES
	CMR-5382, MN136551	*Hipposideridae, Hipposideros cyclops*	73% unidentified adenovirus isolate Aau12186 (MF175106)	ES
	CMR-5395, MN136553	*Hipposideridae, Hipposideros cyclops*	73% unidentified adenovirus isolate Aau12186 (MF175106)	ES
	CMR-5397, MN136555	*Hipposideridae, Hipposideros cyclops*	73% unidentified adenovirus isolate Aau12186 (MF175106)	ES
	CMR-5398, MN136556	*Hipposideridae, Hipposideros cyclops*	73% unidentified adenovirus isolate Aau12186 (MF175106)	ES
	CMR-5634, MN136585	*Pteropodidae, Megaloglossus woermanni*	73% unidentified adenovirus isolate Aau12186 (MF175106)	ES
	CMR-5793, MN136607	*Hipposideridae, Hipposideros cyclops*	72% unidentified adenovirus isolate Aau12186 (MF175106)	SU
	CMR-5998, MN136627	*Hipposideridae, Hipposideros cyclops*	74% unidentified adenovirus isolate Aau12186 (MF175106)	LI
	CMR-6211, MN136628	*Pteropodidae, Myonycteris sp.*	75% unidentified adenovirus isolate Aau12186 (MF175106)	CE
10	CMR-5378, MN136548	*Hipposideridae, Hipposideros cyclops*	72% unidentified adenovirus isolate Aau12186 (MF175106)	ES
	CMR-5795, MN136609	*Hipposideridae, Hipposideros cyclops*	74% unidentified adenovirus isolate Aau12186 (MF175106)	SU
11	CMR-5394, MN136552	*Hipposideridae, Hipposideros cyclops*	70% unidentified adenovirus isolate Aau12186 (MF175106)	ES
12	CMR-5416, MN136558	*Hipposideridae, Hipposideros gigas*	71% Mediterranean horseshoe bat adenovirus 3 strain BS12 (KM043081)	SU
13	CMR-5414, MN136557	*Hipposideridae, Hipposideros gigas*	80% unidentified adenovirus isolate Aau12186 (MF175106)	SU
	CMR-5862, MN136610	*Hipposideridae, Hipposideros gigas*	73% unidentified adenovirus isolate Aau12186 (MF175106)	SU
14	CMR-5603, MN136582	*Pteropodidae, Megaloglossus woermanni*	81% Mediterranean horseshoe bat adenovirus 2 strain BS12 (KM043080)	SU
15	CMR-5206, MN136541	*Hipposideridae, Hipposideros ruber*	81% Kuhl's pipistrelle adenovirus 1 strain 09/09 (KM043100)	ES
	CMR-5213, MN136542	*Hipposideridae, Hipposideros ruber*	81% Kuhl's pipistrelle adenovirus 1 strain 09/09 (KM043100)	ES
	CMR-5499, MN136565	*Hipposideridae, Hipposideros ruber*	81% Kuhl's pipistrelle adenovirus 1 strain 09/09 (KM043100)	SU
	CMR-5901, MN136616	*Hipposideridae, Hipposideros ruber*	82% Kuhl's pipistrelle adenovirus 1 strain 09/09 (KM043100)	SW
16	CMR-5508, MN136567	*Hipposideridae, Hipposideros ruber*	73% marten adenovirus type 2 isolate PM04 (KY753134)	SU
	CMR-5509, MN136568	*Hipposideridae, Hipposideros ruber*	73% marten adenovirus type 2 isolate PM04 (KY753134)	SU
	CMR-5510, MN136569	*Hipposideridae, Hipposideros ruber*	73% marten adenovirus type 2 isolate PM04 (KY753134)	SU

Continued

Table 2. Continued

Species	Isolate/GenBank acc. no.	Host family and species	BLAST N (29/05/18)	Location*
17	CMR-5899, MN136614	*Hipposideridae, Hipposideros ruber*	74% marten adenovirus type 2 isolate PM04 (KY753134)	SW
	CMR-5900, MN136615	*Hipposideridae, Hipposideros ruber*	74% marten adenovirus type 2 isolate PM04 (KY753134)	SW
	CMR-5965, MN136622	*Hipposideridae, Hipposideros ruber*	74% marten adenovirus type 2 isolate PM04 (KY753134)	SW
	CMR-5968, MN136623	*Hipposideridae, Hipposideros ruber*	73% marten adenovirus type 2 isolate PM04 (KY753134)	SW
18	CMR-5504, MN136566	*Hipposideridae, Hipposideros ruber*	75% marten adenovirus type 2 isolate PM04 (KY753134)	SU
	CMR-5715, MN136599	*Hipposideridae, Hipposideros ruber*	73% phocine adenovirus 1 isolate PhAdV-1 (JX244191)	ES
	CMR-5974, MN136624	*Hipposideridae, Hipposideros ruber*	72% phocine adenovirus 1 isolate PhAdV-1 (JX244191)	SW
19	CMR-5691, MN136593	*Pteropodidae, Rousettus aegyptiacus*	98% bat adenovirus isolate BAT0025/Kwale (KY311889)	SW
20	CMR-5690, MN136592	*Pteropodidae, Rousettus aegyptiacus*	79% unidentified adenovirus isolate PgAdV-10 (KC692426)	SW
21	CMR-5704, MN136597	*Pteropodidae, Rousettus aegyptiacus*	79% unidentified adenovirus isolate PgAdV-9 (KC692425)	SW
22	CMR-5735, MN136601	*Vespertilionidae, Neoromicia tenuipinnis*	76% brown long-eared bat adenovirus 1 strain 345/08 (KM043094)	LI
23	CMR-5758, MN136603	*Nycteridae, Nycteris hispida*	76% vespertilionid adenovirus 4 strain 492/08 (KM043107)	SU
24	CMR-5868, MN136611	*Molossidae, Mops condylurus*	73% bottlenose dolphin adenovirus 1 strain Tt11018 (KR024710)	NW
	CMR-5871, MN136612	*Molossidae, Mops condylurus*	73% bottlenose dolphin adenovirus 1 strain Tt11018 (KR024710)	NW
25	CMR-5877, MN136613	*Molossidae, Mops condylurus*	75% Mediterranean horseshoe bat adenovirus 3 strain BS12 (KM043081)	NW
26	CMR-5928, MN136617	*Rhinolophidae, Rhinolophus fumigatus*	84% Mediterranean horseshoe bat adenovirus 3 strain BS12 (KM043081)	N
27	CMR-5703, MN136596	*Pteropodidae, Rousettus angolensis*	79% unidentified adenovirus isolate PgAdV-9 (KC692425)	SW
28	CMR-5736, MN136602	*Vespertilionidae, Neoromicia tenuipinnis*	74% phocine adenovirus 1 isolate PhAdV-1 (JX244191)	LI
29	CMR-5952, MN136619	*Pteropodidae, Eidolon helvum*	100% bat adenovirus isolate BAT0066/Kisii (KY311895)	SW
30	CMR-6226, MN136630	*Rhinolophidae, Rhinolophus alcyone*	75% unidentified adenovirus isolate Aau13092 (MF175107)	CE
	CMR-6227, MN136631	*Rhinolophidae, Rhinolophus alcyone*	74% bottlenose dolphin adenovirus 1 strain Tt11018 (KR024710)	CE
31	CMR-5491, MN136564	*Pteropodidae, Rousettus aegyptiacus*	80% unidentified adenovirus isolate PgAdV-10 (KC692426)	SU
32	CMR-5396, MN136554	*Hipposideridae, Hipposideros cyclops*	71% unidentified adenovirus isolate Aau12186 (MF175106)	ES
	CMR-5792, MN136606	*Hipposideridae, Hipposideros cyclops*	73% Mediterranean horseshoe bat adenovirus 3 strain BS12 (KM043081)	SU
	CMR-5794, MN136608	*Hipposideridae, Hipposideros cyclops*	71% unidentified adenovirus isolate Aau12186 (MF175106)	SU
33	CMR-5687, MN136590	*Pteropodidae, Rousettus aegyptiacus*	80% unidentified adenovirus isolate PgAdV-9 (KC692425)	SW
34	CMR-6243, MN136632	*Pteropodidae, Micropteropus pusillus*	79% unidentified adenovirus isolate PgAdV-11 (KC692427)	N

Continued

Table 2. Continued

Species	Isolate/GenBank acc. no.	Host family and species	BLAST N (29/05/18)	Location*
35	CMR-5983, MN136625	*Pteropodidae, Rousettus aegyptiacus*	76% unidentified adenovirus isolate PgAdV-11 (KC692427)	SW
36	CMR-5471, MN136562	*Pteropodidae, Megaloglossus woermanni*	89% bat mastadenovirus WIV17 (KX961095)	SU
	CMR-5525, MN136571	*Pteropodidae, Megaloglossus woermanni*	89% bat mastadenovirus WIV17 (KX961095)	ES
	CMR-5528, MN136572	*Pteropodidae, Megaloglossus woermanni*	90% bat mastadenovirus WIV17 (KX961095)	ES
	CMR-5563, MN136575	*Pteropodidae, Hypsignathus monstrosus*	89% bat mastadenovirus WIV18 (KX961096)	SU
	CMR-5566, MN136576	*Pteropodidae, Megaloglossus woermanni*	89% bat mastadenovirus WIV17 (KX961095)	SU
	CMR-5591, MN136579	*Pteropodidae, Megaloglossus woermanni*	90% bat mastadenovirus WIV17 (KX961095)	SU
	CMR-5594, MN136580	*Pteropodidae, Megaloglossus woermanni*	90% bat mastadenovirus WIV17 (KX961095)	SU
	CMR-5600, MN136581	*Pteropodidae, Megaloglossus woermanni*	90% bat mastadenovirus WIV17 (KX961095)	SU
	CMR-5609, MN136583	*Pteropodidae, Megaloglossus woermanni*	90% bat mastadenovirus WIV17 (KX961095)	SU
	CMR-5953, MN136620	*Pteropodidae, Megaloglossus woermanni*	90% bat mastadenovirus WIV17 (KX961095)	SW
	CMR-5958, MN136621	*Pteropodidae, Lissonycteris angolensis*	89% bat mastadenovirus WIV17 (KX961095)	SW
	CMR-6265, MN136633	*Pteropodidae, Myonycteris* sp.	91% bat mastadenovirus WIV17 (KX961095)	AD
37	CMR-5661, MN136587	*Pteropodidae, Rousettus aegyptiacus*	89% bat mastadenovirus WIV17 (KX961095)	SU
	CMR-5684, MN136589	*Pteropodidae, Rousettus aegyptiacus*	89% bat mastadenovirus WIV17 (KX961095)	SW
	CMR-5695, MN136594	*Pteropodidae, Rousettus aegyptiacus*	89% bat mastadenovirus WIV17 (KX961095)	SW
	CMR-5788, MN136604	*Pteropodidae, Rousettus aegyptiacus*	90% bat mastadenovirus WIV17 (KX961095)	SU
	CMR-5789, MN136605	*Pteropodidae, Rousettus aegyptiacus*	90% bat mastadenovirus WIV17 (KX961095)	SU
38	CMR-5688, MN136591	*Pteropodidae, Rousettus aegyptiacus*	99% bat adenovirus isolate 1 050 597 (HQ529709)	SW
	CMR-5697, MN136595	*Pteropodidae, Rousettus aegyptiacus*	100% bat adenovirus isolate 1 050 597 (HQ529709)	SW

*Administrative regions in Cameroon: Adamawa (AD), Central (CE), East (ES), Littoral (LI), North (N), North-West (NW), South (SU), South-West (SW).

analysis of 10 full gene bat AdV sequences points in the same direction (File S9). The clusters of virus isolates from hosts of the same family and the relatively consistent separation of clusters of viruses from *Yinpterochiroptera* and *Yangochiroptera* supports the established idea that co-evolution plays an important role. A surprising finding was the composition of several clusters with AdVs isolated from hosts belonging to numerous different genera, and presumed virus species infecting various hosts such as species 2, 4, 6, 9 and 36. Thus, the concept of co-evolution does not follow through to the host genus or species level (Table 2, Fig. 2). We see two possible explanations for this observation. In scenario one, the common ancestor of all bats had a large set of AdVs that was passed on to the diverging species, potentially evolving at a slower rate than the host. In scenario two, the common ancestor of all bats had a rather small set of AdVs that was passed on and diverged with the species, while AdVs were and are to some extent exchanged between bat species (File S10). These are not mutually exclusive, but based on the current data, with detections of some very closely related AdVs in different host species, we consider scenario two more likely to play the major role.

Notably, in GenBank we also identified three AdV isolates where the reported host falls into a different *suborder* than the hosts of related isolates, KY311894 (*Eidolon helvum*), KY311901 (*Otomops martiensseni*) and KY311899 (*Coleura afra*). These three species all form large roosts in trees, caves or ceilings, often in hundreds to tens of thousands of individuals [29]. Those conditions would certainly be ideal to maintain an AdV infection that has successfully jumped a species barrier. Additionally, some of these species occur in caves where multiple species may be present in groups that may be mixed or adjacent. These conditions could potentially lead to a higher diversity of AdVs than in bats that roost in smaller numbers and our data seems to support this. The significantly higher rate of unique AdV species detections among bats roosting in large colonies and the low rate in solitary bats do point in this direction, and so do the observed trends based on the dataset collected for this study and the GenBank dataset that suggest more AdV species per bat species for those roosting in large colonies (File S8). These results have to be interpreted with some caution, since the 37 species we sampled only represent a tiny fraction of Chiroptera diversity, and there likely is some degree of sampling error. Nevertheless, the trend that bat species with preferences for larger roost sizes appear to host a higher diversity of AdVs seems worth following up on. This observation is also consistent with studies finding that larger roost sizes lead to increased transmission of pathogens both in models and with henipaviruses in bats [33, 34].

In the light of the presented findings and observations, and considering what has been discovered regarding evolution and interspecies transmission of CAdV-1, HAdV-4, HAdV-76 and titi monkey AdV [1, 6–9], it is possible that what has previously been interpreted as species specificity in AdVs might in fact be more an effect of lacking contact between species rather than an inability of AdVs to infect other related host species.

In summary, this study contributes significantly to our knowledge about the diversity of AdVs harboured by bats, with up to 44 novel AdV species detected. The results of the analysis give us new insights into the evolution of bat AdVs and AdVs in general, as well as the role of their hosts. These findings suggest that host roost size may play an important role in the epidemiology and AdV evolution as it also does for other viruses, and might also be what has limited some AdVs to single species.

Author contributions
Conceptualization, N. D. W. and C. E. L.; methodology, N. D. W. and C. E. L.; validation, M. L., A. G. and C. E. L.; formal analysis, M. L., A. G. and C. E. L.; investigation, N. F. N., J. L. D. D., V. N. N., J-M. T. and A. G.; resources, J. N.; data curation, M. M. M. M., M. L., A. G. and C. M.; writing – original draft, J. L. D. D., D. J. M. and C. E. L.; writing – review and editing, all authors; visualization, M. L., D. J. M. and C. E. L.; supervision, U. T., M. M. M. M., M. L., A. G., B. S. S., C. M., D. O. J., E. M. R. and C. E. L.; project administration, U. T., J. N., C. M., D. O. J. and E. M. R.; funding acquisition, U. T., D. O. J., A. W. R. and N. D. W.

References
1. Purkayastha A, Ditty SE, Su J, McGraw J, Hadfield TL *et al*. Genomic and bioinformatics analysis of HAdV-4, a human adenovirus causing acute respiratory disease: implications for gene therapy and vaccine vector development. *J Virol* 2005;79:2559–2572.

2. King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ. *Virus Taxonomy: Classification and Nomenclature of Viruses. Ninth Report of the International Committee on Taxonomy of Viruses*. Amsterdam: Elsevier Academic Press; 2012.

3. Berk AJ. Adenoviridae. In: Knipe DM, Howley PM (eds). *Fields Virology*, 6th edn. Philadelphia: Lippincott Williams and Wilkins; 2013. pp. 1704–1731.

4. Wold WSM, Ison MG. Adenoviruses. In: Knipe DM, Howley PM (eds). *Fields Virology*, 6th edn. Philadelphia: Lippincott Williams and Wilkins; 2013. pp. 1732–1767.

5. Benkö M, Harrach B. Molecular evolution of adenoviruses. *Curr Top Microbiol Immunol* 2003;272:3–35.

6. Chen EC, Yagi S, Kelly KR, Mendoza SP, Tarara RP *et al*. Cross-species transmission of a novel adenovirus associated with a fulminant pneumonia outbreak in a new world monkey colony. *PLoS Pathog* 2011;7:e1002155.

7. Balboni A, Verin R, Morandi F, Poli A, Prosperi S *et al*. Molecular epidemiology of canine adenovirus type 1 and type 2 in free-ranging red foxes (Vulpes vulpes) in Italy. *Vet Microbiol* 2013;162:551–557.

8. Dehghan S, Seto J, Liu EB, Walsh MP, Dyer DW *et al*. Computational analysis of four human adenovirus type 4 genomes reveals molecular evolution through two interspecies recombination events. *Virology* 2013;443:197–207.

9. Dehghan S, Seto J, Liu EB, Ismail AM, Madupu R *et al*. A zoonotic adenoviral human pathogen emerged through genomic recombination among human and nonhuman simian hosts. *J Virol* 2019;93:e00564-19.

10. Lange CE, Niama FR, Cameron K, Olsen SH, Aime Nina R *et al*. First evidence of a new simian adenovirus clustering with human mastadenovirus F viruses. *Virol J* 2019;16:147.

11. Zheng X-Y, Qiu M, Ke X-M, Guan W-J, Li J-M *et al*. Detection of novel adenoviruses in fecal specimens from rodents and shrews in southern China. *Virus Genes* 2016;52:417–421.

12. Diffo J, Ndze VN, Ntumvi NF, Takuo J-M, Mouiche MMM *et al*. DNA of diverse adenoviruses detected in Cameroonian rodent and shrew species. *Arch Virol* 2019;164:2359–2366.

13. Kumakamba C, N'Kawa F, Kingebeni PM, Losoma JA, Lukusa IN *et al*. Analysis of adenovirus DNA detected in rodent species from the Democratic Republic of the Congo indicates potentially novel adenovirus types. *New Microbes New Infect* 2020;34:100640.

14. Vidovszky M, Kohl C, Boldogh S, Görföl T, Wibbelt G *et al*. Random sampling of the central European bat fauna reveals the existence of numerous hitherto unknown adenoviruses. *Acta Vet Hung* 2015;63:508–525.

15. Tan B, Yang XL, Ge X-Y, Peng C, Liu H-Z *et al*. Novel bat adenoviruses with low G+C content shed new light on the evolution of adenoviruses. *J Gen Virol* 2017;98:739–748.

16. Sonntag M, Mühldorfer K, Speck S, Wibbelt G, Kurth A. New adenovirus in bats, Germany. *Emerg Infect Dis* 2009;15:2052–2055.

17. Li Y, Ge X, Zhang H, Zhou P, Zhu Y *et al*. Host range, prevalence, and genetic diversity of adenoviruses in bats. *J Virol* 2010;84:3889–3897.

18. Jánoska M, Vidovszky M, Molnár V, Liptovszky M, Harrach B *et al*. Novel adenoviruses and herpesviruses detected in bats. *Vet J* 2011;189:118–121.

19. Raut CG, Yadav PD, Towner JS, Amman BR, Erickson BR *et al*. Isolation of a novel adenovirus from *Rousettus leschenaultii* bats from India. *Intervirology* 2012;55:488–490.

20. Hu D, Zhu C, Wang Y, Ai L, Yang L *et al*. Virome analysis for identification of novel mammalian viruses in bats from Southeast China. *Sci Rep* 2017;7:10917.

21. Ogawa H, Kajihara M, Nao N, Shigeno A, Fujikura D *et al*. Characterization of a novel bat adenovirus isolated from straw-colored fruit bat (*Eidolon helvum*). *Viruses* 2017;9:371.

22. Iglesias-Caballero M, Juste J, Vázquez-Morón S, Falcon A, Aznar-Lopez C *et al*. New adenovirus groups in Western Palaearctic bats. *Viruses* 2018;10:443.

23. Kohl C, Vidovszky MZ, Mühldorfer K, Dabrowski PW, Radonić A *et al*. Genome analysis of bat adenovirus 2: indications of interspecies transmission. *J Virol* 2012;86:1888–1892.

24. Podgorski II, Pantó L, Földes K, de Winter I, Jánoska M *et al*. Adenoviruses of the most ancient primate lineages support the theory on virus-host co-evolution. *Acta Vet Hung* 2018;66:474–487.

25. Wellehan JF, Johnson AJ, Harrach B, Benkö M, Pessier AP *et al*. Detection and analysis of six lizard adenoviruses by consensus primer PCR provides further evidence of a reptilian origin for the atadenoviruses. *J Virol* 2004;78:13366–13369.

26. Townzen JS, Brower AV, Judd DD. Identification of mosquito blood-meals using mitochondrial cytochrome oxidase subunit I and cytochrome b gene sequences. *Med Vet Entomol* 2008;22:386–393.

27. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A *et al*. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 2012;61:539–542.

28. Rosevear DR. *The Bats of West Africa*. London: British Museum (Natural History); 1965.

29. Monadjem A, Taylor PJ, Cotterill W, Schoeman MC. *Bats of Southern and Central Africa: a Biogeographic and Taxonomic Synthesis*. Johannesburg: Wits University Press; 2010. p. 596.

30. Wilson DE, Mittermeier RA. *Handbook of the Mammals of the World, vol. 9, Bats*. Barcelona: Lynx; 2019.

31. Conow C, Fielder D, Ovadia Y, Libeskind-Hadas R. Jane: a new tool for the cophylogeny reconstruction problem. *Algorithms Mol Biol* 2010;5:16.

32. Parker J, Rambaut A, Pybus OG. Correlating viral phenotypes with phylogeny: accounting for phylogenetic uncertainty. *Infect Genet Evol* 2008;8:239–246.

33. Laughlin AJ, Hall RJ, Taylor CM. Ecological determinants of pathogen transmission in communally roosting species. *Theor Ecol* 2019;12:225–235.

34. Rahman SA, Hassan L, Epstein JH, Mamat ZC, Yatim AM *et al*. Risk factors for Nipah virus infection among pteropid bats, Peninsular Malaysia. *Emerg Infect Dis* 2013;19:51–60.