

1 **Diversification of mammalian deltaviruses by host shifting**

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19

20 **Abstract**

21 Hepatitis delta virus (HDV) is an obligate hyper-parasite that increases the severity of hepatitis B
22 virus (HBV) in humans. The origins of HDV and the mechanisms through which it and related
23 animal deltaviruses diversify are unknown. We report the epidemiology and evolutionary history
24 of new mammal-infecting deltaviruses. Despite geographic under-representation in over 348
25 terabases of globally-distributed RNA sequence data from mammals, deltaviruses originated
26 exclusively from the Americas, infecting bats, rodents and a cervid. Phylogenetic analyses
27 revealed multiple host shifts among mammalian orders. Consistent absence of HBV-like viruses
28 in two deltavirus-infected bat species indicated acquisitions of novel helper viruses during the
29 divergence of animal and human-infecting deltaviruses. Our analyses support an American
30 zoonotic origin of HDV and show that deltaviruses can diversify by host shifting despite
31 dependence on unrelated viruses.

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51 Main Text

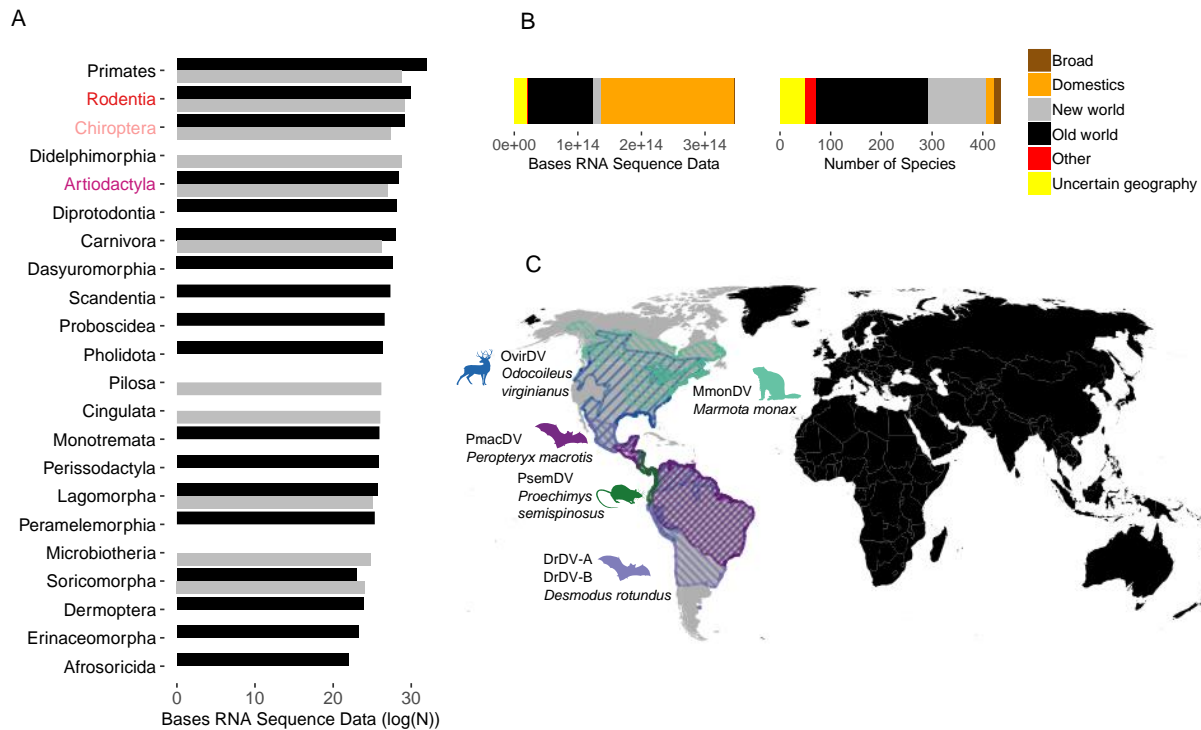
52 Hepatitis delta virus (HDV) is a globally-distributed human pathogen which causes the most
53 severe form of viral hepatitis in an estimated 20 million people. Unlike typical viruses, HDV is
54 an obligate ‘satellite’ virus that is replicated by diverse host cells, but requires the envelope of an
55 unrelated ‘helper’ virus (classically hepatitis B virus, HBV, family *Hepadnaviridae*) for cellular
56 entry, egress and transmission (1). The peculiar life history of HDV together with its lack of
57 sequence homology to known viral groups has made the evolutionary origins of HDV a
58 longstanding puzzle. Geographic associations of most HDV genotypes point to an Old World
59 origin. Yet, historical explanations of the mechanistic origin of HDV spanned from emergence
60 from the mRNA of a HBV-infected human (2) to ancient evolution from viroids (circular, single-
61 stranded RNA pathogens of plants) (3). More recently, discoveries of HDV-like genomes in
62 vertebrates and invertebrates (4-7) overturned the decades-long belief that deltaviruses
63 exclusively infect humans. These discoveries also suggested new models of deltavirus evolution,
64 in which viruses either co-specified with their hosts over ancient timescales or possess an
65 unrecognized capacity for host shifting which would imply their potential to emerge in novel
66 species.

67 Efforts to distinguish competing evolutionary hypotheses for deltaviruses have been
68 precluded by the remarkably sparse distribution of currently-known HDV-like agents across the
69 animal tree of life. Single representatives are reported from arthropods (Subterranean termite,
70 *Schedorhinotermes intermedius*), fish (a pooled sample from multiple species), birds (a pooled
71 sample from 3 duck species, *Anas gracilis*, *A. castanea*, *A. superciliosa*), reptiles (Common boa,
72 *Boa constrictor*) and mammals (Tome’s spiny rat, *Proechimys semispinosus*), and only two are
73 known from amphibians (Asiatic toad, *Bufo gargarizans*; Chinese fire belly newt, *Cynops*
74 *orientalis*) (4-7). Most share minimal homology with HDV, even at the protein level (< 25%),
75 frustrating robust phylogenetic re-constructions of evolutionary histories (Fig. S1). On the one
76 hand, the distribution of deltaviruses may reflect rare host shifting events among divergent taxa.
77 Alternatively, reliance on untargeted metagenomic sequencing (a relatively new and selectively
78 applied tool) to find novel species may mean that the distribution of deltaviruses in nature is
79 largely incomplete (8, 9). Additional taxa could reveal ancient co-speciation of HDV-like agents
80 with their hosts or evidence for host shifting.

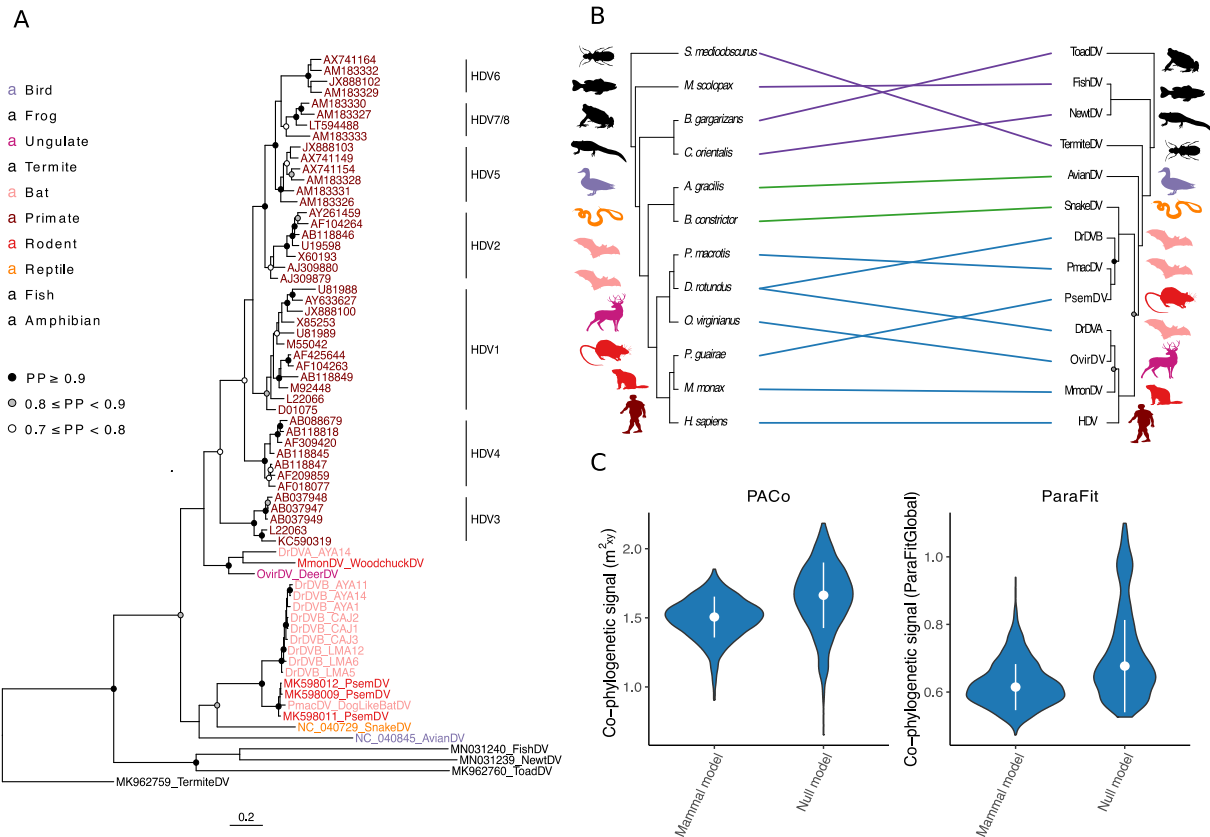
81 We sought to fill gaps in the evolutionary history of mammalian deltaviruses, the group
82 most likely to clarify the origins of HDV. We used a two-pronged approach (Materials and
83 Methods). First, we used data from Serratus, a newly developed bioinformatic platform which
84 screens RNA sequences from the NCBI Short Read Archive (SRA) for similarity to known
85 viruses and which is described by Edgar *et al.* (10). We focused on search results from 96,695
86 transcriptomic and metagenomic datasets, comprising 348 terabases of RNA sequences from 403
87 species across 24 mammalian orders (22 terrestrial, 2 aquatic; Data S1). Although domesticated
88 animals comprised the largest single fraction of the dataset (67.2%), remaining data were from a
89 variety of globally-distributed species (Fig 1A,B). Our second search was prompted by our
90 earlier detection of uncharacterized deltavirus-like sequences in a neotropical bat (11) and
91 evidence of under-representation in the volume of neotropical bat data in the SRA (Fig. 1A). We
92 therefore carried out metagenomic sequencing of 23 frugivorous, insectivorous, nectarivorous,
93 and sanguivorous bat species from Peru, using 59 samples available within our laboratory (Table
94 S1). All datasets containing sequences with significant protein homology to deltaviruses were
95 subjected to *de novo* genome assembly.

96 Searches revealed five deltaviruses spanning three mammalian orders: Artiodactyla
97 (N=1), Chiroptera (N=3), and Rodentia (N=1; Fig. 1C). Strikingly, despite over-representation of
98 Old World-derived data by factors of 4.3 (Artiodactyla), 5.8 (Chiroptera), 2.1 (Rodentia), all new
99 mammalian deltaviruses originated from North and South American species (Supplementary
100 Results Section 1, Fig. 1A,C). Chiropteran deltaviruses included two genotypes from common
101 vampire bats (*Desmodus rotundus*) which shared only 48.4-48.6% genome-wide nucleotide (nt)
102 identity (hereafter, DrDV-A and DrDV-B; Fig. S1). A third deltavirus was identified in a liver
103 transcriptome (accession SRR7910143; (12)) from a lesser dog-like bat (*Peropteryx macrotis*)
104 from Mexico (PmacDV), but was more closely related to recently described deltaviruses from
105 Tome's spiny rat from Panama (PsemDV; (7)), sharing 95.9-97.4% (amino acid) and 93.0-95.7%
106 (nt) identity. Additional genomes were recovered from transcriptomes derived from the pedicle
107 tissue of a white-tailed deer (*Odocoileus virginianus*; OvirDV; accession SRR4256033; (13))
108 and from a captive-born Eastern woodchuck (*Marmota monax*; MmonDV; accession
109 SRR2136906; (14)). Bioinformatic screens recovered additional reads matching each genome in
110 related datasets (either different individuals from the same study or different tissues from the
111 same individuals), suggesting active infections (Table S2). All genomes had lengths 1669-1771

112 nucleotides, high intramolecular base pairing, and contained genomic and antigenomic
 113 ribozymes characteristic of deltaviruses. The DrDV-A and DrDV-B genomes are more fully
 114 characterized in Fig S2, Fig S3, Table S3 and Supplementary Results Section 2. The other
 115 genomes and a case study on MmonDV infections in animals inoculated with woodchuck
 116 hepatitis virus are described by Edgar *et al.* (10).



117
 118 **Fig. 1. Geographic and taxonomic distribution of mammalian datasets and novel**
 119 **deltaviruses.** (A) The host and geographic distribution of metagenomic and transcriptomic
 120 datasets searched for novel deltaviruses, note the log scale. Colors indicate orders (red =
 121 Rodentia; pink = Chiroptera; Purple = Artiodactyla). (B) Stacked bar charts show the volume of
 122 mammalian datasets in units of RNA bases and the number of species searched, separated by
 123 species geography. Additional segments describe widely distributed domesticated animals
 124 (Domestics), datasets with genus-level metadata from broadly-distributed genera (Broad),
 125 datasets from cell lines or with taxonomic information only at the Class level (Other), and those
 126 which had no geographic range data available (Uncertain geography, mostly aquatic mammals).
 127 (C) Host distributions of newly discovered and recently reported deltaviruses, color coded by
 128 mammalian species (Data from IUCN). All except PsemDV were discovered through our search.
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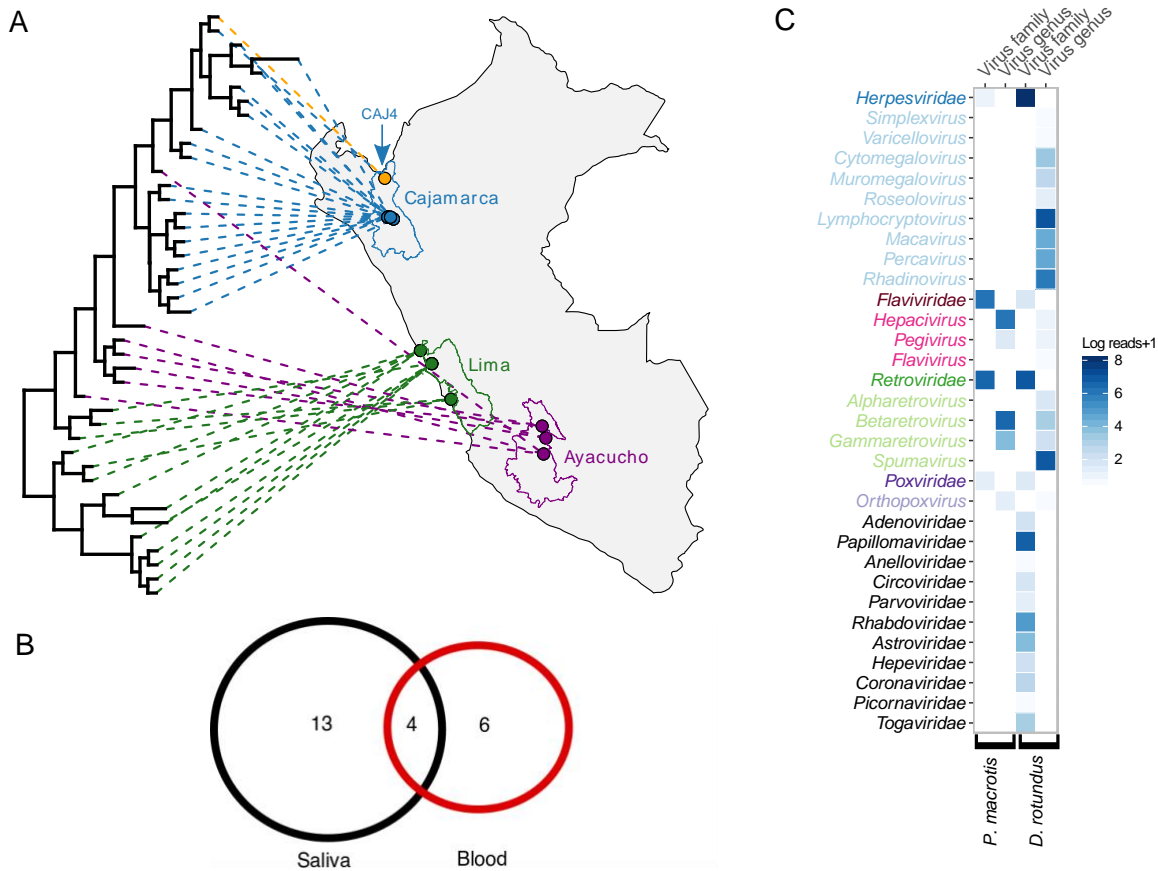


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 132 **Fig. 2. Evolutionary history of deltaviruses reveals host shifts among mammals.** (A) Bayesian
 133 phylogeny of a 192 amino acid alignment of the DAG. Ingroup taxa including mammal, snake and
 134 avian deltaviruses are colored by order; other HDV-like taxa are shown in black. (B) Co-phylogeny
 135 depicting connections between the consensus deltavirus phylogeny from StarBeast and the host
 136 tree (from TimeTree.org). Links are colored according to subsets of data used in co-phylogenetic
 137 analyses; all taxa (purple + green + blue), ingroup (green + blue), or mammal (blue). Host taxa
 138 are: *Schedorhinotermes medioobscurus*, *Macroramphosus scolopax*, *Bufo gargarizans*, *Cynops*
 139 *orientalis*, *Anas gracilis*, *Boa constrictor*, *Peroptryx macrotis*, *Desmodus rotundus*, *Odocoileus*
 140 *virginianus*, *Proechimys guirae*, *Marmota monax*, and *Homo sapiens*. (C) Absence of
 141 phylogenetic dependence of the mammalian deltavirus phylogeny on the host phylogeny. Violin
 142 plots show distributions of test statistics from two co-phylogenetic approaches across 1,000
 143 posterior trees relative to null models, along with medians and standard deviations. For PACo,
 144 higher values would indicate greater phylogenetic dependence; for ParaFit, lower values. Both
 145 approaches rejected a global model of co-speciation ($P > 0.05$).
 146

147 Phylogenetic analysis of the small delta antigen (DAG) protein sequences using MrBayes
 148 (Fig. 2A) and a multi-species coalescent model in StarBeast (Fig. 2B) revealed multiple putative
 149 host shifts within the evolutionary history of mammalian deltaviruses. For instance, vampire bat
 150 deltaviruses were paraphyletic, suggesting at least two independent incursions into this species.
 151 Specifically, DrDV-A formed a clade with OvirDV and MmonDV (posterior probability, $PP =$

152 0.99/0.80 in MrBayes and StarBeast respectively) which was basal to HDV ($PP = 0.65/0.81$),
153 while DrDV-B shared a most recent common ancestor with PmacDV and PsemDV ($PP = 1/1$).
154 Rodent-associated deltaviruses (MmonDV and PsemDV) were also highly divergent and
155 paraphyletic. Consequently, co-phylogenetic analyses using 1,000 randomly sampled topologies
156 from StarBeast failed to reject independence of mammal and deltavirus phylogenies, consistent
157 with a model of diversification by host shifting (Fig. 2B,C). Analyses of all deltavirus-host pairs
158 (i.e. including highly divergent HDV-like agents) and an ‘ingroup’ clade containing mammalian
159 along with avian and snake deltaviruses revealed somewhat greater dependence of the deltavirus
160 phylogeny on the host phylogeny (Fig. S4). However, statistical significance varied across co-
161 phylogenetic approaches and topological incongruences were evident among non-mammals,
162 excluding co-speciation as the sole diversification process, even in deeper parts of the co-
163 evolutionary history (Fig. S5, Supplementary Results Section 3).

164 Having extended the mammalian host range of deltaviruses to neotropical bats, we
165 subsequently explored the transmission dynamics, host range, and candidate helper virus
166 associations within this group through a field study in three regions of Peru (Fig. 3A). Out of 240
167 *D. rotundus* saliva samples from 12 bat colonies collected in 2016-2017, RT-PCR targeting the
168 DA_g detected DrDV-A in single adult female from one of the two metagenomic pools that
169 contained this genotype (bat ID 8299, site AYA14, Table S4, S5). In contrast, DrDV-B was
170 detected in 17.1% of *D. rotundus* saliva samples (colony-level prevalence: 0-35%). Prevalence
171 varied neither by region of Peru (Likelihood ratio test; $\chi^2 = 3.21$; d.f. = 2; $P = 0.2$) nor by bat age
172 or sex (binomial generalized linear mixed model, Age: $P = 0.38$; Sex: $P = 0.87$), suggesting
173 geographic and demographic ubiquity of infections. Given that vampire bats subsist on blood,
174 deltavirus sequences encountered in bat saliva might represent contamination from infected prey.
175 A small set of blood samples screened for DrDV-A ($N = 60$, including bat 8299) were negative.
176 However, 6 out of 41 bats that were DrDV-B negative and 4 out of 18 bats with DrDV-B in
177 saliva also contained DrDV-B in blood samples (Fig. 3B). In the 4 individuals with paired saliva
178 and blood samples, DA_g sequences were identical, supporting systemic infections. Significant
179 spatial clustering of DrDV-B sequences at both regional and bat colony levels further supported
180 local infection cycles driven by horizontal transmission among vampire bats (Fig. 3A; Table S6).



181

182 **Fig. 3. Transmission biology and candidate helper viruses for bat deltaviruses.** (A) Bayesian
 183 phylogeny of a 214 nt alignment of DrDV-B DAg projected onto vampire bat capture locations
 184 in Peru. Lines and points are colored by administrative regions. Site CAJ4, where the *C.*
 185 *perspicillata* sequence was detected, is depicted in orange. (B) DrDV-B detections in saliva and
 186 blood. Numbers represent individual bats; the four bats in the center had genetically identical
 187 DrDV-B sequences in saliva and blood. (C) Mammal-infecting viral communities are shown for
 188 the *P. macrotis* liver transcriptome which contained PmacDV and combined *D. rotundus* saliva
 189 metagenomes. Viral taxa are colored by family, with lighter shades indicating genera within
 190 families, for overlapping viral families in both bat species. Viral families only present in one bat
 191 species are shown in black. Candidate helpers for OvirDV and MonDV are shown in Fig. S8.
 192

193 Given the evolutionary evidence for deltavirus host shifts, we hypothesized that spillover
 194 infections might also occur at detectable frequencies in sympatric neotropical bats. Among 87
 195 non-*D. rotundus* bats captured in or outside of *D. rotundus*-occupied roosts, RT-PCR detected
 196 deltavirus RNA in the saliva of a single Seba's short-tailed bat (*Carollia perspicillata*; N=31
 197 individuals; Fig. S6). This result was unlikely attributable to erroneous bat species assignment or
 198 laboratory contamination (Supplementary Results, Section 4). The partial DAg recovered from

199 the *C. perspicillata* was identical to a DrDV-B strain collected from a vampire bat in the same
200 roost (CAJ4; Fig. 3A). Given the rapid evolution expected in deltaviruses (ca. 10^{-3}
201 substitutions/site/year), genetic identity is most parsimoniously explained as horizontal
202 transmission from *D. rotundus* to *C. perspicillata*, which was followed by an absence of (or
203 short-lived) transmission among *C. perspicillata* at the time of sampling (15). This finding
204 therefore demonstrates cross-species transmission on ecological timescales, a defining
205 prerequisite for evolutionary diversification of deltaviruses through host shifting.

206 Finally, we evaluated whether bat deltaviruses use hepadnavirus helpers akin to HDV (1).
207 Consistent with a previous study, PCR screens of DrDV-positive and negative saliva (N=54) and
208 blood samples (N=119), found no evidence of hepadnaviruses in vampire bats (16). To rule out
209 divergent hepadnaviruses missed by PCR, we next used a bioinformatic pipeline to characterize
210 viral communities in the metagenomic and transcriptomic datasets from deltavirus-infected bat
211 species (Materials and Methods). Hepadnaviruses were again absent from all datasets (Fig. 3C).
212 Together with the finding that all three bat-infecting deltavirus genomes lacked the farnesylation
213 site thought to facilitate acquisition of the hepadnaviral envelope (Supplementary Results,
214 Section 2), use of hepadnavirus helpers by bat deltaviruses seems unlikely. To identify
215 alternative plausible candidates, we quantified the abundance (approximated by sequence reads)
216 of viral taxa that overlapped between the two deltavirus-infected bat species, *P. macrotus* and *D.*
217 *rotundus*. In the *P. macrotus* liver which contained PMacDV, reads from hepaciviruses
218 (*Flaviviridae*) spanned a complete viral genome and outnumbered all other viral genera with the
219 exception of Betaretroviruses, whose abundance reflects endogenization in the host genome (Fig.
220 3C). Lower, but detectable, hepacivirus abundance in vampire bats may reflect the tissue tropism
221 of these viruses or pooling of samples from multiple individuals. Intriguingly, hepaciviruses
222 experimentally mobilize HDV *in vitro* and were found in 26 out of 30 PsemDV-infected rats (7,
223 17). Reads matching Poxviridae formed small contigs in both libraries (*P. macrotus*: 229-1386
224 nt; *D. rotundus*: 358 nt) and particularly in *D. rotundus*, could not be excluded as false positives.
225 Although non-opportunistic sampling is required to decisively identify the helpers of bat
226 deltaviruses, existing evidence points to hepaciviruses as top contenders, perhaps using
227 alternative enveloping mechanisms to HDV.

228 Unlike conventional pathogens (e.g., viruses, bacteria, protozoans), the obligatory
229 dependence of deltaviruses on evolutionarily independent helpers creates a barrier to cross-

230 species transmission that might be expected to promote host specificity. Data to test this
231 hypothesis have been unavailable until now. Our study demonstrates transmission of deltaviruses
232 among highly divergent mammalian orders on both ecological and evolutionary timescales.

233 Deltavirus host shifts could conceivably arise through several processes. Mobilization by
234 non-viral micro-organisms (e.g., intra-cellular bacteria) is conceivable but has never been
235 observed. Unaided spread through a yet undefined mechanism is also possible. However, given
236 that the best-studied deltavirus (HDV) depends on viral envelopes to complete its life cycle and
237 that conserved genomic features in related deltaviruses suggest a similar life history strategy,
238 helper virus mediated host shifting is the most reasonable expectation. We and others have
239 excluded hepadnavirus helpers for PmacDV, DrDVs, and PsemDV, yet natural HDV infections
240 consistently involve HBV (1, 7). In light of this and the evidence presented here for host shifts
241 among mammals, the contemporary HDV-HBV association must have arisen through acquisition
242 of the hepadnaviral helper somewhere along the evolutionary divergence separating human and
243 other mammal-infecting deltaviruses. Evidence that deltaviruses can exploit diverse enveloped
244 viruses experimentally adds further weight to this conclusion (17, 18). As several new
245 mammalian deltaviruses were detected with hepacivirus and poxvirus co-infections, either
246 simultaneous host shifts of deltaviruses and helpers, or preferential deltavirus shifting among
247 host species that are already infected with compatible helpers are plausible. Conclusively
248 identifying the helper associations of novel mammalian deltaviruses and their evolutionary
249 relationships will be crucial to disentangling these possibilities.

250 A limitation of our study was that the species in which novel deltaviruses were
251 discovered were presumed to be definitive hosts (i.e. capable of sustained horizontal
252 transmission). Consequently, some putative host shifts detected in our co-phylogenetic analysis
253 may represent short-lived transmission chains in novel hosts or singleton infections, analogous to
254 our observation of DrDV-B in *C. perspicillata*. For example, PmacDV from the Lesser dog-like
255 bat clustered within the genetic diversity of PsemDV from rats, but infected two out of three
256 individual bats analyzed, suggesting a recent cross-species transmission event followed by some
257 currently unknown amount of onward transmission. Irrespective of the long-term outcomes of
258 index infections, our results unequivocally support the conclusion that deltaviruses can transmit
259 between divergent mammalian orders. The global distribution of deltavirus positive and negative
260 datasets provides additional, independent evidence for host shifts. Even allowing for sub-

261 detection due to variation in infection prevalence and dataset quality, deltaviruses should have
262 been more widespread across the mammalian phylogeny than we observed (ca. 1% of species
263 analyzed) if they co-specified with their hosts. Moreover, the three deltavirus-infected
264 mammalian orders occur in both the New and Old Worlds, yet non-human deltaviruses occurred
265 exclusively in the Americas. Sampling biases cannot readily explain this pattern. By most
266 metrics of sequencing effort, Old World mammals were over-represented each deltavirus-
267 infected mammalian order, including at finer continental scales (Supplementary Results, Section
268 1). Despite the large scale of our search, we evaluated < 10% of mammalian species. We
269 therefore anticipate further discoveries of mammalian deltaviruses. Crucially, however, new
270 viruses could not re-unite the paraphyletic rodent and bat deltaviruses or resolve widespread
271 incongruence between mammal and deltavirus phylogenies, making our conclusions on host
272 shifting robust.

273 The origin of HDV has been a longstanding mystery thwarted by the absence of closely
274 related deltaviruses. The addition of 6 new mammalian deltaviruses by ourselves and others
275 allowed us to re-evaluate this question (7, 10). The pervasiveness of host shifting among
276 deltaviruses and our discovery of a clade of mammalian deltaviruses basal to HDV (albeit with
277 variable support depending on phylogeny, $PP = 0.64-0.81$) strongly points to a zoonotic origin.
278 Although the exact progenitor virus remains undiscovered, the exclusive detection of mammalian
279 deltaviruses in the New World species supports an “out of the Americas” explanation for origin
280 and global spread of HDV (Fig. 1). The basal placement of the highly divergent Amazonian
281 HDV genotype (HDV-3) within the phylogeny lends further credence to this scenario. Though
282 circumstantial, the earliest records of HDV are from the Amazon basin in the 1930s (19). The
283 greater diversity of HDV genotypes outside of the Americas – long argued to support an Old
284 World origin – may instead reflect diversification arising from geographic vicariance within
285 human populations.

286 Our results show that deltaviruses jump between mammalian host species through an
287 unusual process that most likely requires parasitizing evolutionarily independent viruses. Since
288 satellite viruses in general and HDV in particular tend to alter the pathogenesis and
289 transmissibility of their helpers (20), our findings imply the potential for deltaviruses to act as
290 host-switching virulence factors that could alter the progression of viral infections in multiple
291 host species. Constraints on future host shifts are likely to differ from those of conventional

292 animal pathogens. Specifically, given the broad cellular tropism of deltaviruses, interactions with
293 helpers would likely be more important determinants of cross-species transmission than
294 interactions with their hosts (17, 18). Consequently, anticipating future host shifts requires
295 understanding the determinants and plasticity of deltavirus and helper compatibility along with
296 the ecological factors that enable cross-species exposure.

297

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313 Genbank (accessions MT649206- MT649209). PmacDV, OvirDV and MmonDV genome
314 sequences are available at <https://serratus.io/access>. Peruvian bat metagenomes are available in
315 ENA project PRJEB35111. Scripts used for bioinformatic analyses are available on GitHub
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319 original draft (LMB, DGS); all authors contributed to review and editing of the manuscript.

320 **Supplementary Materials:**

321 Materials and Methods

322 Supplementary Results

323 Figures S1-S8

324 Tables S1-S6

325 Supplementary References

326

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Supplementary Materials for

Diversification of mammalian deltaviruses by host shifting

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393 **Materials and Methods**

394 **1. Virus discovery**

395 **a. Serratus**

396 The Serratus platform was used to search published mammalian metagenomic and transcriptomic
397 sequence datasets available in the NCBI Short Read Archive (SRA). Briefly, Serratus uses a
398 cloud computing infrastructure to perform ultra-high throughput alignment of publicly available
399 SRA short read datasets to viral genomes of interest (1). Due to the exceptional computational
400 demands of this search and mutual interest between ourselves and another research team,
401 Serratus searches were designed by both teams and carried out at the protein and nucleotide
402 levels by Edgar *et al.* (1). Query sequences for Serratus searches included all HDV genotypes
403 and all deltavirus-like genomes which were publicly available at the time of the search (July
404 2020) along with representative genomes from DrDV-A and DrDV-B, which our team had
405 already discovered. Results were shared among the two teams who subsequently pursued
406 complementary lines of investigation. Novel mammalian deltaviruses were discovered using
407 nucleotide (OvirDV) and amino acid level (Mmon DV and PmacDV) searches of the mammalian
408 SRA search space.

409 **b. Neotropical bat metagenomic sequencing**

410 Total nucleic acid was extracted from archived saliva swabs from Neotropical bats on a
411 Kingfisher Flex 96 automated extraction machine (ThermoFisher Scientific) with the Biosprint
412 One-for-all Vet Kit (Qiagen) using a modified version of the manufacturer's protocol as
413 described previously (2). Ten pools of nucleic acids from vampire bats and other bat species
414 were created for shotgun metagenomic sequencing (Table S1). Eight pools comprised samples
415 from bats in the same genus (2-10 individuals per pool depending on availability of samples, 30
416 μ L total nucleic acid per individual). The CAJ1 vampire bat pool from (3) which contained
417 deltavirus reads was included as a sequencing control. The final pool ("Rare species") comprised
418 8 other bat species that had only one individual sampled each. Pools were treated with DNase I
419 (Ambion) and purified using RNAClean XP beads (Agencourt) following (2). Libraries were
420 prepared using the SMARTer Stranded Total RNA-Seq Kit v2 - Pico Input Mammalian
421 (Clontech) and sequenced on an Illumina NextSeq500 at The University of Glasgow Polyomics
422 Facility. Samples were bioinformatically processed for viral discovery as described previously

423 (2), with a slight modification to the read trimming step to account for shorter reads and a
424 different library preparation kit.

425 **c. DrDV genome assembly and annotation**

426 DrDV genomes were assembled using SPAdes (4) and refined by mapping cleaned reads back to
427 SPAdes-generated contigs within Geneious v 7.1.7 (5). Regions of overlapping sequence at the
428 ends of genomes due to linear *de novo* assemblies of circular genomes were resolved manually.
429 Genome circularity was confirmed based on the presence of overlapping reads across the entire
430 circular genome of both DrDVs. The amino acid sequence of the small delta antigen protein
431 (DAg) was extracted from sequences using getorf (6). Other smaller identified open reading
432 frames did not exhibit significant homology when evaluated by protein blast against Genbank.
433 Nucleotide sequences of full deltavirus genomes and amino acid sequences of DAg were aligned
434 along with representative sequences from other deltaviruses using the E-INS-i algorithm in
435 MAFFT v 7.017 (7). Genetic distances as percent identities were calculated based on an
436 untrimmed full genome alignment of 2,321bp and an untrimmed delta antigen alignment of
437 281aa. Protein domain homology of the DAg was analyzed using Hhpred (8). Ribozymes were
438 identified manually by examining the region upstream of the delta antigen open reading frame
439 where ribozymes are located in other deltavirus genomes (9, 10). RNA secondary structure and
440 self-complementarity were determined using the webservers for mFold (11) and RNAstructure
441 (12). We found no evidence of recombination in nucleotide alignments of DrDV DAg according
442 to the program GARD (13) on the Datamonkey webserver (14). Genome assembly and
443 annotation of PmacDV, OvirDV, and MmondDV are described in (1).

444 **d. Evaluation of deltavirus positive cohorts**

445 To establish that deltaviruses were likely to be actively infecting hosts in which they were
446 detected, and not laboratory contamination or incidental detection of environmentally derived
447 RNA, we searched for evidence of deltavirus infections in additional samples from the various
448 studies that detected full genomes. Samples included sequencing libraries derived from both
449 different individuals in the same study and different tissues from the same individuals. Searches
450 used bwa (15) to map raw reads from deltavirus positive cohorts to the corresponding novel
451 deltavirus genomes which had been detected in those same cohorts. Genome remapping was
452 performed for all vampire bat libraries, two other *Peropteryx macrotis* libraries and all other
453 Neotropical bat species sequenced in the same study (16) and other pooled tissue samples

454 sequenced by RNASeq from *Odocoileus virginianus* in the same study (17). Results are shown
455 in Table S2. Deltavirus reads from additional individuals and timepoints from the *Marmota*
456 *monax* study (18) are described in (1).

457 **e. Global biogeographic analysis of deltavirus presence and absence**

458 To characterize the global distribution of mammal-infecting deltaviruses, we used the metadata
459 of each SRA accession queried in the Serratus search to identify the associated host. We focused
460 primarily on the mammalian dataset, which was generated by the SRA search query
461 (“Mammalia”[Organism] NOT “Homo sapiens”[Organism] NOT “Mus musculus”[Organism])
462 AND (“type_rnaseq”[Filter] OR “metagenomic”[Filter] OR “metatranscriptomic”[Filter]) AND
463 “platform illumina”[Properties]. All analyses were performed in R version 3.5.1 ((19). We
464 removed libraries with persistent errors which had not completed in the Serratus search. For
465 remaining libraries, when host identification information was available to the species level, we
466 matched Latin binomial species names to Pantheria, a dataset which contains the centroids of
467 mammalian geographic distributions, and used an R script to assign species to continents using
468 these geographic data (20). Subspecies present in scientific names of SRA meta-data were re-
469 assigned to species level and recently updated binomial names were changed to match the
470 Pantheria dataset. Due to the fact that mammalian taxonomic data in Pantheria date to 2005,
471 some former Orders which are no longer in use (e.g. Soricomorpha) appear in our data but are
472 not expected to affect the results of analyses. Forty-eight species whose centroids occurred in
473 water bodies were assigned to continents by manually inspecting species ranges. Widely-
474 distributed domesticated animals, datasets with genus-level metadata from broadly-distributed
475 genera, datasets from cell lines or with taxonomic information only at the Class level, and those
476 which had no geographic range data available (mostly aquatic mammals) were searched by
477 Serratus, but excluded from geographic comparisons. We quantified geographic and taxonomic
478 biases in our dataset both in units of bases of RNA sequenced and number of species
479 investigated.

480 Although most mammalian metagenomic and transcriptomic libraries were captured in
481 the mammalian search, we also examined datasets from SRA search queries for vertebrates,
482 metagenomes, and viromes to ensure that all relevant libraries were captured in our measures of
483 search effort. For these datasets, we removed libraries with persistent errors and calculated
484 search effort as bases of RNA sequenced across the three orders in which deltaviruses were

485 discovered, removing libraries named for specific viral taxa which may have been enriched for
486 these taxa and therefore do not represent a likely source of novel viruses. As these libraries
487 lacked species-level meta-data (hence their exclusion from the mammalian search above), we
488 could not systematically calculate number of species in these datasets. Additional search queries
489 are available at <https://github.com/ababaian/serratus/wiki/SRA-queries>.

490 **2. Phylogenetic analyses**

491 **a. Bayesian phylogeny using MrBayes**

492 Phylogenetic analysis was performed on complete DA_g amino acid sequences to place
493 mammalian deltaviruses relative to HDV and other described deltaviruses. Representative
494 sequences from each clade of HDV and other previously published DVs were aligned with
495 DrDVs (sequences generated by RT-PCR, described in Methods section 3b), PmacDV, OvirDV
496 and MmonDV using the E-INS-i algorithm and JTT2000 scoring matrix of MAFFT within
497 Geneious. Large delta antigen sequences from HDV were trimmed manually to small delta
498 antigen length, and the alignment was further trimmed in trimal using the automated1 setting to a
499 final length of 192aa. The best substitution model (JTTDCMut+F+G4) was determined using
500 ModelFinder (21) within IQTree 2 (22). Bayesian phylogenetic analysis was performed using the
501 most similar model available within MrBayes (JTT+F+G4). The analysis was run for 5,000,000
502 generations and sampled every 2,500 generations, with the first 500 trees discarded as burn-in to
503 generate the consensus tree.

504 **b. Bayesian phylogeny using StarBeast**

505 We used StarBeast to generate a species-level phylogeny for the co-phylogenetic analysis, using
506 the same amino acid alignment of complete DA_g which was used in the MrBayes analysis.
507 StarBeast is typically used with multi-locus sequence data from multiple individuals per species
508 but can also be applied to single gene alignments (23). Notably, a preliminary analysis suggested
509 this approach was more conservative than a constant effective size coalescent model in BEAST
510 which substantially inflated posterior probabilities on nodes across the tree relative to the
511 MrBayes analysis (Fig. 2A). The StarBeast multi-species coalescent analysis was carried out as
512 two duplicate runs of 50 million generations (sampling every 5000 generations) in BEAST2,
513 using the JTT+G substitution model, the linear with constant root model for the species tree
514 population size, and a Yule speciation model. Combined log files were assessed for convergence

515 and effective sample size values >200 using TRACER. Twenty percent of trees were discarded
516 as burn-in prior to generating the consensus tree.

517 **c. Co-phylogeny**

518 Co-phylogenetic analyses were performed in R using PACo (24, 25) and ParaFit (26, 27).

519 Analyses were performed on three subsets of matched host-deltavirus data: all taxa, ingroup taxa
520 (mammals, bird and snake) and mammals only. Host datasets consisted of distance matrices
521 derived from a TimeTree phylogeny (timetree.org). For metagenomic libraries which contained
522 individuals of multiple species (AvianDV and FishDV), one host was selected for inclusion
523 (*Anas gracilis* and *Macroramphosus scolopax*, respectively). Host data were not available in
524 TimeTree for two species in which deltaviruses were discovered (*Proechimys semispinosus* and
525 *Schedorhinotermes intermedius*), so available congeners were substituted (*Proechimys guairae*
526 and *Schedorhinotermes medioobscurus*, respectively). Virus datasets consisted of distances
527 matrices from posterior species trees generated in StarBeast. Co-phylogeny analyses performed
528 using virus distance matrices derived from posterior MrBayes trees, pruned to contain only
529 relevant taxa, yielded qualitatively similar results. For both analyses, the principal coordinates
530 analysis of distance matrices was performed with the ‘cailliez’ correction. Since units of branch
531 length differed between host and virus trees, all distance matrices were normalized prior to co-
532 evolutionary analyses. To account for phylogenetic uncertainty in the evolutionary history of
533 deltaviruses, analyses were carried out using 1,000 trees randomly selected from the posterior
534 distribution of the Bayesian phylogenetic analyses (separately for StarBeast and MrBayes). Due
535 to uncertain placement of HDV3 in both phylogenies, one representative of HDV was randomly
536 selected for each iteration. For each tree, we calculated summary statistics (see below) describing
537 the dependence of the deltavirus phylogeny on the host phylogeny. *P*-values were estimated
538 using 1,000 permutations of host-virus associations.

539 For PACo analyses, the null model selected was r0, which assumes that virus phylogeny
540 tracks the host phylogeny. Levels of co-phylogenetic signal were evaluated as the median global
541 sum of squared residuals (m_{2xy}) and mean significance (*P*-values), averaged over the 1,000
542 posterior trees. Empirical distributions were compared to null distributions for each dataset. For
543 ParaFit analyses, the levels of co-phylogenetic signal in each dataset were evaluated as the
544 median sum of squares of the fourth corner matrix (ParaFitGlobal) and mean significance (*P*-
545 values), averaged over 1,000 posterior trees. ParaFit calculates the significance of the global

546 host-virus association statistic by randomly permuting hosts in the host-virus association matrix
547 to create a null distribution. Since Parafit does not provide this distribution to users, we
548 approximated it for visualization by manually re-estimating the global host-virus association
549 statistic for 1,000 random permutations of hosts in the host-virus association matrix. Phylogenies
550 and co-phylogenies were visualized in R using the packages ‘ape’ (27), ‘phangorn’ (28),
551 ‘phytools’ (29), and ‘ggtree’ (30).

552 **3. Deltaviruses in Neotropical bats**

553 **a. Capture and sampling of wild bats**

554 To examine DrDV prevalence in vampire bats, we studied 12 sites in three departments of Peru
555 between 2016-2017 (Fig. 3A). Age and sex of bats were determined as described previously (2).
556 Saliva samples were collected by allowing bats to chew on sterile cotton-tipped wooden swabs
557 (Fisherbrand). Blood was collected from vampire bats only by lancing the propatagial vein and
558 saturating a sterile cotton-tipped wooden swab with blood. Swabs were stored in 1 mL
559 RNALater (Ambion) overnight at 4°C before being transferred to dry ice and stored in -70°C
560 freezers.

561 Bat sampling protocols were approved by the Research Ethics Committee of the
562 University of Glasgow School of Medical, Veterinary and Life Sciences (Ref081/15), the
563 University of Georgia Animal Care and Use Committee (A2014 04-016-Y3-A5), and the
564 Peruvian Government (RD-009-2015-SERFOR-DGGSPFFS, RD-264-2015-SERFOR-
565 DGGSPFFS, RD-142-2015-SERFOR-DGGSPFFS, RD-054-2016-SERFOR-DGGSPFFS).

566 **b. RT-PCR and sequencing of blood and saliva samples**

567 Primers were designed to screen bat saliva and blood samples for a conserved region of the DA_g
568 protein of DrDV-A (236bp) and DrDV-B (231bp), by hemi-nested and nested RT-PCR
569 respectively (Table S4). Alternative primers were designed to amplify the complete DA_g for
570 DrDV-A (707bp) and DrDV-B (948bp) using a one-step RT-PCR (Table S4). cDNA was
571 generated from total nucleic acid extracts using the Protoscript II First Strand cDNA synthesis kit
572 with random hexamers; RNA and random hexamers were heated for 5 minutes at 65°C then
573 placed on ice. Protoscript II reaction mix and Protoscript II enzyme mix were added to a final
574 concentration of 1x, and the reaction was incubated at 25°C for 5 minutes, 42°C for 15 minutes,
575 and 80°C for 5 minutes. PCR was performed using Q5 High-Fidelity DNA Polymerase (NEB).
576 Each reaction contained 1x Q5 reaction buffer, 200 μM dNTPs, 0.5 μM each primer, 0.02 U/μL

577 Q5 High Fidelity DNA polymerase and either 2.5 μ L cDNA or 1 μ L Round 1 PCR product.
578 Reactions were incubated at 98°C for 30 seconds, followed by 40 cycles of 98°C for 10 seconds,
579 61-65°C for 30 seconds (or 58-60°C for 30 seconds for the complete DA_g), 72°C for 40 seconds,
580 and a final elongation step of 72°C for 2 minutes. PCR products of the correct size were
581 confirmed by re-amplification from cDNA or total nucleic acid extracts and/or Sanger
582 sequencing (Eurofins Genomics).

583 **c. Bat species confirmation**

584 We confirmed the morphological species assignment of the *C. perspicillata* individual in which
585 DrDV-B was detected by sequencing cytochrome B. Cytochrome B was amplified from the same
586 saliva sample in which DrDV-B was detected using primers Bat 05A and Bat 04A (31) and
587 GoTaq Green Master Mix (Promega) according to the manufacturer's instructions, and the
588 resulting product was Sanger sequenced (Eurofins Genomics) then evaluated by nucleotide blast
589 against Genbank.

590 **d. Genetic diversity and distribution of DrDV-B**

591 To examine relationships among DrDV-B sequences, Bayesian phylogenetic analysis was
592 performed on a 214bp fragment of the DA_g. Sequences from saliva and blood of 41 *D. rotundus*
593 and saliva from one *C. perspicillata* were aligned using MAFFT within Geneious. Duplicate
594 sequences originating from the blood and saliva of the same individuals were removed.
595 Alignments were trimmed using Trimal (32) with automated1 settings, and the best model of
596 sequence evolution was determined using jModelTest2 (33). Phylogenetic analysis was
597 performed using MrBayes 3.6.2 (34) with the GTR+I model. The analysis was run for 4,000,000
598 generations and sampled every 2,000 generations, with the first 1,000 trees removed as burn-in.
599 The association between phylogenetic relationships and location at both the regional and colony
600 level was tested using BaTS (35) with 1,000 posterior trees and 1,000 replicates to generate the
601 null distribution.

602 **e. Statistical analyses of DrDV-B**

603 We modeled the effects of age and sex on DrDV-B presence in saliva using a binomial
604 generalized linear mixed model (GLMM) in the package lme4 (36) in R. Age (female/male) and
605 sex (adult/subadult) were modeled as categorical variables, with site included as a random effect.
606 We also evaluated differences in DrDV-B prevalence between regions of Peru using a binomial

607 generalized linear model (GLM), and used the *Anova* function of the *car* package (37) to
608 calculate the likelihood ratio χ^2 test statistic.

609 **4. Identifying candidate helper viruses for mammalian deltaviruses**

610 **a. Hepadnavirus screening in vampire bats**

611 We tested samples for the presence of bat hepadnavirus as a candidate helper virus to DrDV.
612 DNA from saliva and blood samples was screened for HBV-like viruses using pan-
613 *Hepadnaviridae* primers (HBV-F248, HBV-R397, HBV-R450a, HBV-R450b; Table S4) and
614 PCR protocols (38). We used a plasmid carrying a 1.3-mer genome of human HBV that is
615 particle assembly defective but replication competent as a positive control.

616 **b. Bioinformatic screening of published metagenomic datasets**

617 We performed comprehensive virus discovery using an in-house bioinformatic pipeline (2) on
618 sequence datasets containing deltaviruses to identify candidate helper viruses. Datasets analyzed
619 included all vampire bat datasets (22 from (2), 46 from (3)), *P. macrotis* datasets (SRR7910142,
620 SRR7910143, SRR7910144), *O. virginianus* datasets (SRR4256025-SRR4256034), and *M.*
621 *monax* datasets (SRR2136906, SRR2136907). Briefly, after quality trimming and filtering, reads
622 were analyzed by blastx using DIAMOND (39) against a RefSeq database to remove bacterial
623 and eukaryotic reads. Remaining reads were then *de novo* assembled using SPAdes (4) and
624 resulting contigs were analyzed by blastx using DIAMOND against a non-redundant (NR)
625 protein database (40). KronaTools (41) and MEGAN (42) were used to visualize and report
626 taxonomic assignments.

627

628 **Supplementary Results**

629 **1. Evaluation of continent level geographic biases in Serratus data**

630 We sought to confirm that the sole detection of mammalian deltaviruses in the Americas in three
631 different mammalian orders was unlikely to have arisen from sampling biases in the RNA
632 sequence datasets that we analyzed. The main text illustrates geographic patterns at broad scales
633 and shows that New World species were under-represented relative to Old World species,
634 indicating that sampling biases were unlikely to explain the absence of deltaviruses from Old
635 World species. However, we also examined biases at finer geographic and taxonomic scales,
636 focusing on the mammalian SRA search query dataset for which there was species and continent
637 level information. At the continent level, data volumes (in bases of RNA sequenced) declined

638 from Asia (6.35e13), Africa (2.89e13), South America (7.99e12), North America (4.93e12),
639 Europe (4.86e12), to Australia (3.75e12) (Fig S7). This implies that Africa (the previously
640 assumed origin of HDV) has more than double the data of the Americas combined. Similar
641 patterns were evident within the deltavirus-infected mammalian orders. For Artiodactyla, North
642 and South American datasets were ranked 3rd and 5th respectively among the 5 continents which
643 had sequence data. Although there was less Artiodactyla data from Africa than North America,
644 Asia (1st) and Europe (2nd) had 2 and 1.6-fold more data than the Americas combined. For bats,
645 North and South American datasets were ranked 5th and 6th among the 6 continents with data,
646 and Europe (1st) and Africa (2nd) had 2.5 and 1.2-fold more data than datasets combined across
647 the Americas. For rodents, North America datasets were ranked 2nd, while South American
648 datasets were ranked 5th out of the 5 continents with data, but Asia (1st) had 1.1-fold more data
649 than the Americas combined and Africa had 3.6 times more data than South America.

650 We also examined potential biases in search effort by number of species sequenced per
651 continent. There were equal or fewer species of Artiodactyla sequenced in North (N = 5) and
652 South America (N=1) compared to Europe (N = 5), Asia (N = 8) and Africa (N = 14). Similarly,
653 rodent species sampled from Old World continents (N = 48; Europe [N = 8], Africa [N = 14],
654 and Asia [N = 26]) outnumbered those in the New World (N = 33; North America [N = 21],
655 South America [N = 12]. Neither Rodentia nor Artiodactyla datasets were searched from
656 Australia. In contrast, there were more bat species in North and South American datasets (N = 4
657 and 42, respectively) compared to Asia (N = 22), Africa (N = 5), Europe (N = 2), and Australia
658 (N =1) leading to a slight bias toward New World bats (46 species vs 30 Old World species).
659 Consequently, in the ‘mammalian’ dataset, New World bats were more numerous at the species
660 level, but had fewer individuals tested per species and/or less sequencing depth per species.

661 We further examined datasets generated by related search queries (vertebrate, metagenome
662 and virome) which included some libraries from mammals that were excluded from the
663 ‘mammalian’ dataset (see Materials and Methods, Section 1e). The vertebrate dataset contained
664 no matches to the three mammalian Orders of interest (Rodentia, Artiodactyla, Chiroptera). For
665 Rodentia, the metagenome dataset contained 380 libraries (9.7e7 bases) identified as “mouse
666 metagenome” and the virome dataset contained 171 libraries (1e11 bases) which were identified
667 as “mouse gut metagenome” or “rodent metagenome” or “Rattus” or “rat gut metagenome”.
668 These could not be assigned geographic provenance and likely represented laboratory animals.

669 For Artiodactyla, the virome dataset contained 317 libraries (1e11 bases) which were identified
670 as “pig gut metagenome” or “pig metagenome” or “bovine gut metagenome”. As these libraries
671 likely derived from either experimental or domestic animals, we conclude that additional data
672 from these two Orders are unlikely to influence geographic or taxonomic bias. For Chiroptera,
673 there were a total of 25 libraries (2.4e10 bases) identified as “bat metagenome”. The vast
674 majority (N=24) were from Old World bats. Eleven derived from a study of bat rotaviruses
675 (PRJNA562472) which of which 10 were collected from Old world locations (Ghana, Bulgaria)
676 and one from New World (Costa Rica). Two libraries were bat viral metagenomes generated
677 from samples collected in South Africa (SRR5889194; SRR5889129), and twelve libraries were
678 generated from bats sampled in China (PRJNA379515). There were 14 bat species analyzed in
679 all of these libraries, of which eight were not included in the mammalian SRA dataset, bringing
680 the total number of Old World bat species to 38. Therefore, by the metric of number of species,
681 New World bats remained slightly over-represented (38 versus 46 species) though as mentioned
682 above, Old World bat derived datasets were sequenced more comprehensively and covered a
683 larger number of continents.

684 Overall, across the three orders in which we detected deltaviruses, fewer species were studied
685 in North and South American datasets (85 species) compared to those from Africa, Asia, Europe
686 and Australia (105 species) and the total volume of RNA sequenced was 2.7 times greater for
687 Old world species (1.64e13 bases RNA) than New world species (6.02e12). We therefore
688 conclude that the exclusive presence of deltaviruses in American mammals is unlikely to
689 represent geographic biases in our datasets.

690 **2. Large delta antigen in novel mammalian deltaviruses**

691 In HDV, the large delta antigen protein (L-HDAg) is produced by RNA editing of the UAG stop
692 codon to include 19 additional aa (43) and contains a farnesylation site which interacts with
693 HBV (44). The DrDV-B DAg from the genome from bat colony CAJ1 terminated in UAG,
694 which if edited similarly to HDV would generate a putative L-DAg containing an additional 28
695 aa (Fig. S3). In contrast, DrDV-B DAg from the two other bat colonies from which genomes
696 were sequenced (LMA6 and AYA11), as well as DrDV-A DAg, terminated in a UAA stop
697 codon so would not appear to be similarly edited, although it is possible to extend the open
698 reading frames through frameshifting (9). Importantly, no putative vampire bat L-DAg generated

699 through either RNA editing or frameshifting contained a farnesylation site. PmacDV, OvirDV,
700 and MmonDV also did not contain apparent L-DAG extensions or farnesylation sites (*I*).

701 **3. Co-phylogenetic results across posterior distributions of trees from two Bayesian** 702 **searches and two different co-phylogeny analyses**

703 Consensus topologies differed slightly between the phylogenetic analyses of deltaviruses using a
704 multi-species coalescent model in StarBeast and a coalescent model in MrBayes, particularly in
705 relation to the termite-associated deltavirus-like agent and avian deltavirus (compare Fig. 2A and
706 2B). We therefore repeated our co-phylogenetic analysis using 1,000 trees from the MrBayes
707 analysis to verify that our conclusions were robust to this topological inconsistency. Analyses
708 performed using virus distance matrices derived from posterior MrBayes trees were congruent
709 with those in StarBeast.

710 In the broadest analyses of all taxa, results differed slightly among co-phylogenetic
711 analyses. Specifically, PACo analyses supported the dependence of the deltavirus phylogeny on
712 the host phylogeny (StarBeast trees: $m_{2xy} = 0.57$ (standard deviation = 0.48- 0.66); $m_{2xy_null} =$
713 1.27 (1.11- 1.44); $P = 0.01$; MrBayes trees: ($m_{2xy} = 0.53$ (0.46- 0.6); $m_{2xy_null} = 1.26$ (1.1- 1.42); P
714 $= 0.002$). However, ParaFit analyses supported independence of the virus and host phylogenies
715 based on both StarBeast trees (ParaFitGlobal = 0.72 (standard deviation = 0.59- 0.86); $P = 0.09$)
716 and MrBayes trees (ParaFitGlobal = 0.63 (0.52- 0.75); $P = 0.07$). Similarly, for ingroup taxa
717 (mammalian, avian and snake deltaviruses), PACo detected evidence of co-phylogeny using both
718 sets of trees (Starbeast: $m_{2xy} = 0.81$ (0.66- 0.96); $m_{2xy_null} = 1.3$ (1.14- 1.46); $P = 0.02$; MrBayes:
719 $m_{2xy} = 0.76$ (0.69- 0.83); $m_{2xy_null} = 1.28$ (1.1- 1.46); $P = 0.01$), while ParaFit analyses with
720 StarBeast trees (ParaFitGlobal = 0.8 (0.7- 0.89); $P = 0.12$) and MrBayes trees (ParaFitGlobal =
721 0.68 (0.62- 0.75); $P = 0.09$) found no significant support for co-phylogeny.

722 All analyses of mammalian deltaviruses failed to reject the null hypothesis of independence
723 of phylogenies. These results were consistent when with both StarBeast and MrBayes trees in
724 PACo (StarBeast: $m_{2xy} = 1.51$ (1.36- 1.65); $m_{2xy_null} = 1.66$ (1.43- 1.9); $P = 0.28$; MrBayes: m_{2xy}
725 $= 1.16$ (1.07- 1.25); $m_{2xy_null} = 1.22$ (0.97- 1.48); $P = 0.35$), as well as in ParaFit (StarBeast:
726 ParaFitGlobal = 0.61 (0.55- 0.68); $P = 0.52$; MrBayes: ParaFitGlobal = 0.49 (0.44- 0.54); $P =$
727 0.5).

728 In summary, both PACo and ParaFit analyses of StarBeast and MrBayes trees showed no
729 support for co-phylogeny in the mammalian dataset. Including more divergent host-virus pairs

730 increased support for co-phylogeny in the all taxa and ingroup datasets, with these results being
731 statistically significant in PACo analyses but not in ParaFit analyses. Inconsistent support for
732 phylogenetic independence at broader scales may reflect variation in the sensitivity of different
733 analyses to detect phylogenetic congruence which occurs in only a subset of branches. For
734 example, the non-ingroup deltavirus-like agents formed a polytomy of long branches and were
735 found in the most divergent hosts from mammals, which may have inflated co-phylogenetic
736 signal (Fig. S5). Regardless, given the consistent evidence against a co-speciation model among
737 mammals and incongruences observed among other taxa in the consensus topologies, these
738 findings illustrate that a model of co-speciation alone cannot explain the evolutionary
739 relationships of deltaviruses and their hosts.

740 **4. Putative cross-species transmission of DrDV-B to a frugivorous bat.**

741 The detection of a vampire bat associated deltavirus in a frugivorous bat (*Carollia perspicillata*)
742 is strongly suggestive of cross-species transmission but might also arise through mis-assignment
743 of bat species in the field or contamination of samples during laboratory processing. To exclude
744 the possibility of host species mis-identification, we confirmed morphological species
745 assignment by sequencing Cytochrome B from the same saliva sample in which we amplified
746 deltavirus (see Methods), which showed 99.49% identity with a published *C. perspicillata*
747 sequence in Genbank (Accession AF511977.1). Laboratory contamination was minimized by
748 processing all samples through a dedicated PCR pipeline with a one directional workflow. PCR
749 reagents are stored and master mixes prepared in a laboratory that is DNA/RNA free, and which
750 cannot be entered after going into any other lab. Field collected samples from bats are extracted
751 and handled in a room strictly used for clinical samples which cannot be entered after going in
752 any other lab aside from the master mix room. To further exclude laboratory contamination, we
753 independently amplified the *C. perspicillata* deltavirus product from two separate batches of
754 cDNA. We used only round 1 primers of a nested PCR to avoid detecting trace amounts of
755 potential contamination; in vampire bats only 68% of individuals deemed positive after round 2
756 were also positive in round 1. Furthermore, in the laboratory, samples from other bat species
757 were handled separately from samples collected from vampire bats, with extractions and PCRs
758 being performed on different days. As discussed in the main text, the absence of genetic
759 divergence from sympatric strains in *D. rotundus* indicates limited or no onward transmission of
760 DrDV-B in *C. perspicillata*. Whether the *C. perspicillata* sustained an actively replicating

761 infection is uncertain, although detection in a single round of PCR (which was true for only 68%
762 of DrDV-positive vampire bats) implies an intensity of infection which could suggest DrDV
763 replication in the recipient host, though this would require further testing to confirm. Definitively
764 resolving the extent of DrDV-B replication could be achieved using a quantitative RT-PCR
765 targeting the DrDV antigenome. Such assays do not currently exist and after the confirmatory
766 testing above, in addition to metagenomic sequencing, we unfortunately would no longer have
767 sufficient RNA available from the *C. perspicillata* bat to run such a test if it were available. In
768 summary, we are confident that the individual in which the deltavirus was detected is a *C.*
769 *perspicillata* and we believe the most likely explanation to be cross-species transmission in
770 nature, though whether this represents an active infection remains uncertain.

771 **5. Candidate helper viruses of OvirDV and MmonDV**

772 We also examined viral communities in *O. virginianus* and *M. monax* libraries for candidate
773 helpers. Given that MmonDV was detected in animals experimentally inoculated with
774 Woodchuck Hepatitis Virus (WHV, a Hepadnavirus), these libraries were unsurprisingly
775 dominated by WHV, but also contained reads matching to Herpesviridae, Flaviviridae,
776 Poxviridae and Retroviridae (Figure S8). *O. virginianus* libraries contained Poxviridae,
777 Retroviridae, and Herpesviridae reads. Consequently, reads matching to Poxviridae were
778 detected in libraries for all deltavirus hosts which were studied here (DrDV-A, DrDV-B,
779 PmacDV, MmonDV, OvirDV), although reads were less abundant than other viral taxa and
780 could not always be decisively ruled out as false positives. Although there is no experimental
781 evidence that Poxviruses can produce infectious deltavirus particles, this ecological association
782 may be worth considering in future studies of mammalian deltaviruses.

783 **Supplementary Figures**

784

	U81989	AB118846	AB118845	AM183326	AM183329	AM183333	AM183327	AB037948	OvirDV	MmonDV	DrDV-A	PmacDV	MKS98009	DrDV-B	NC_040729	NC_040845	MN031240	MN031239	MK962760	MK962759
U81989_HDV1		73.5	72.3	69.6	68.3	68.9	71.2	60.4	57.8	51.5	53	46.8	46.8	47.5	41.8	37.2	31.9	30.7	28.1	30.1
AB118846_HDV2	73		76	74.7	74.1	71.5	72.5	62.5	58.7	52.6	54.5	47.5	47.4	48.2	42	37.5	33.2	31.7	28.5	30.2
AB118845_HDV4	74.4	75.7		74.3	72.6	71.5	72.9	63.1	61.5	53.4	56.1	48	48	49	41.8	37.4	32.9	30.4	27.8	29.9
AM183326_HDV5	70.9	77.1	75.3		74.7	72.6	72.1	61	58.6	51.2	55.1	47.8	47.8	48	42.4	37.8	32.2	31.5	28.3	30.7
AM183329_HDV6	69.3	72	69.6	76.3		71.9	72.3	62.4	59.2	52.8	54.5	47.9	47.8	48.1	41.8	35.7	32.1	31.4	27.7	31.2
AM183333_HDV7	68	72.6	70.2	77.3	72.6		75.4	60.9	58.7	51.1	52.1	46.9	46.9	47.3	42.8	36.3	31.2	31	27.9	30.1
AM183327_HDV8	68.9	72.4	75.7	79.9	75.8	75.4		61.5	58.9	52.2	53.6	47.6	46.9	47.5	42.1	35.7	31.1	31.1	26.3	30.4
AB037948_HDV3	66.8	63.2	66.1	65.7	64.6	63.3	64.7		55	50.6	52	46.5	46.1	46.7	41.4	35.7	30.1	29.8	26.7	30.1
OvirDV	61	64.1	69.1	65.1	66.1	62.7	65.6	63		55.8	61.6	47	47.4	47.1	42.7	35.8	31.2	31.1	25.7	28.2
MmonDV	55.4	57.4	60.3	56.4	54.7	54.5	54.9	59.4	67		56.9	45.8	46.2	45.9	43.5	33.7	28.5	29.4	23.4	27.4
DrDV-A	62.6	63.6	64.4	62.6	61.4	60.1	60.5	60.4	74.7	70.1		47	47.4	47.6	43.3	36.3	29	28.5	24.4	27.5
PmacDV	54.3	53.8	55.6	52.3	52.6	52.9	54.3	53.7	57.7	55.1	56.1		95.4	71.2	44.4	33.7	29	30	24.7	26.2
MKS98009_PsemDV	54.8	53.8	56.1	52.8	54.2	53.4	54.8	54.7	58.2	55.1	56.1	96.9		72.6	43.8	33.7	29.4	30.1	24.2	26.3
DrDV-B_CAJ1	52.8	51.8	53.1	51.8	51.6	49.9	52.3	54.2	56.1	55.1	56.7	79.6	81.1		43.7	34.5	29.7	29.6	24.7	27.3
NC_040729_SnakeDV	50	47.2	47.7	46.2	47.8	45.9	45.2	47.8	50.8	50.3	51.8	53	54.5	53		34.2	28.7	27.1	23.2	26.2
NC_040845_AvianDV	40.7	36.2	39.4	36.7	35.7	37.9	36.2	38.9	37.2	39.9	38.3	34.7	35.3	37.9	34.9		25.7	30.4	24.9	25.4
MN031240_FishDV	19.8	18.9	20.9	19.9	20.4	18.5	20.4	19.9	23.4	22.5	20.9	19.3	19.3	21.3	17.1	16.1		24.5	22.1	24.4
MN031239_NewtDV	21.8	20.6	19.7	20.6	18.9	19	20.2	20.2	19.6	18.8	20.1	16.9	17.3	17.8	16.7	21.1	14.7		22.9	24.3
MK962760_ToadDV	20.7	24.6	20.9	20.9	20.4	21	19.3	19.9	18.2	20.4	20.9	20.2	20.2	18.6	18.4	20.1	16.8	16.2		19.8
MK962759_TermiteDV	20	18.2	20.1	21.6	19.7	22.2	21.1	21.1	20.1	19.7	21.6	19	21	20.5	18.8	21.5	10.8	14.9	10.4	

785

786

787 **Fig. S1.** Genetic distances matrices showing representative deltavirus sequences with percent
 788 nucleotide identities between genomes (upper triangle) and percent amino acid identities between
 789 complete DAg sequences (lower triangle). Darker shading indicates higher percentage identity
 790 between two deltaviruses

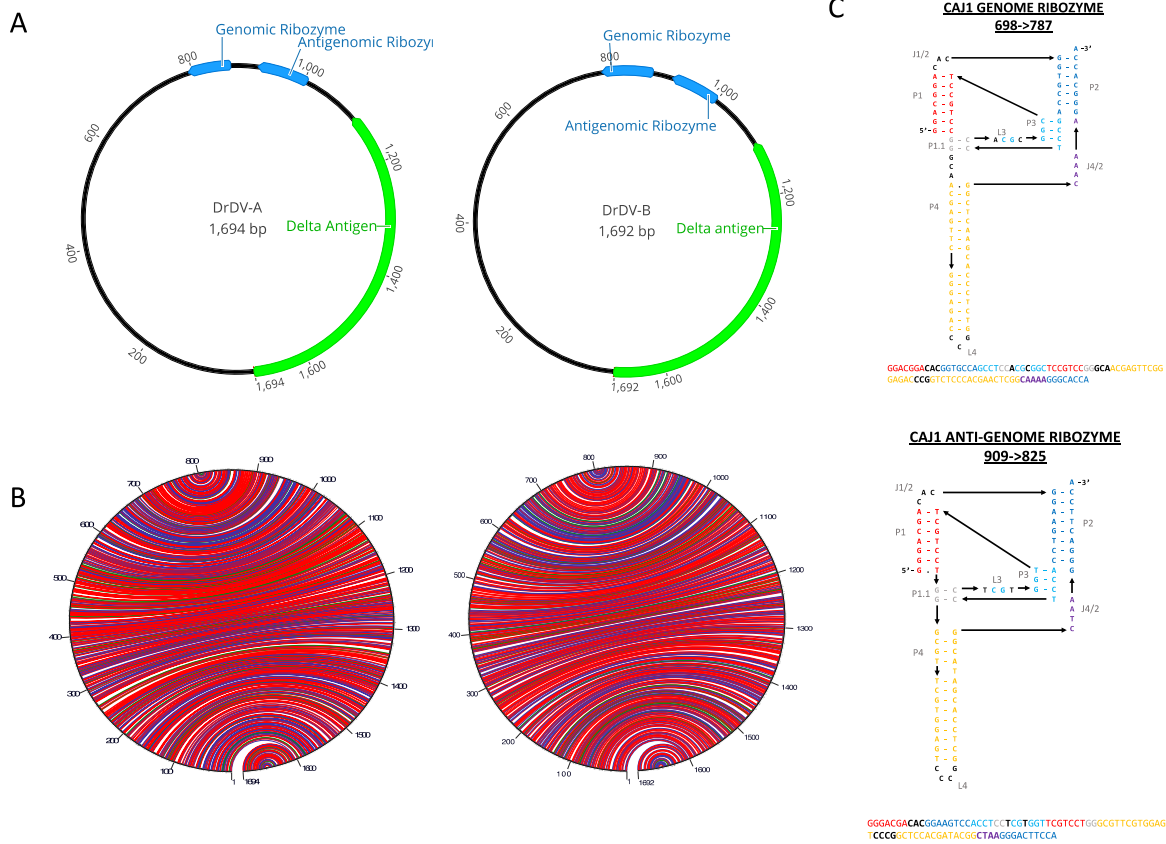
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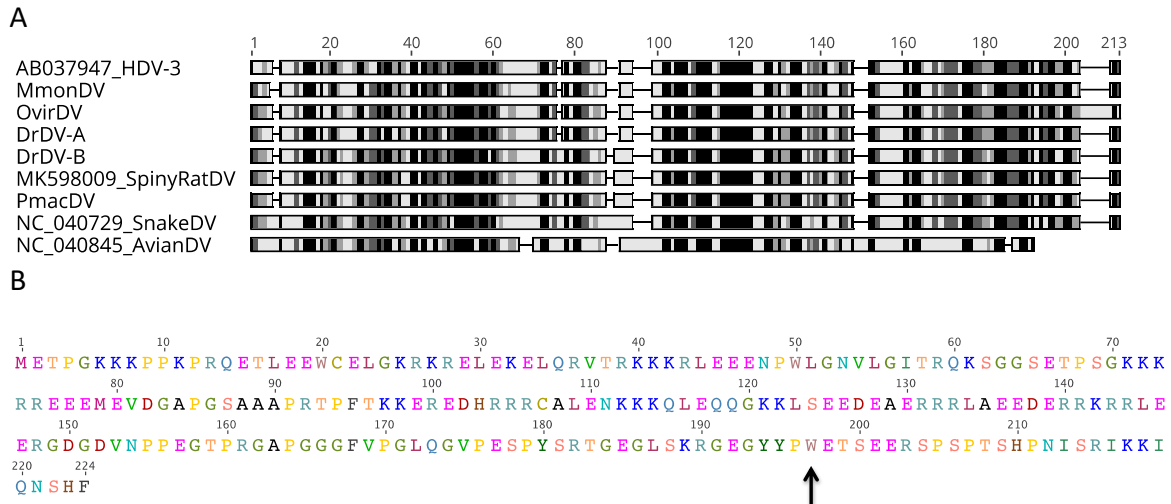
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796

797 **Fig. S2. DrDV genomes exhibit characteristics common to deltaviruses.** (A) The locations of
 798 the delta antigen open reading frame (green) and genomic/antigenomic ribozymes (blue) are
 799 shown along the circular genomes of DrDV-A and DrDV-B (CAJ1 shown as an example). (B)
 800 Intramolecular base pairing for DrDVs depicted as lines connecting points on the circular genome
 801 – G-C pairs are red, A-U pairs are blue, G-U pairs are green, other pairs are yellow. (C) Genomic
 802 and antigenomic ribozyme secondary structures are shown along with genome location for genome
 803 CAJ1. Complementary regions are shown in the same color, and structures are depicted in the style
 804 of Webb & Luptak to facilitate comparison with ribozymes from previous studies (9, 10, 45).

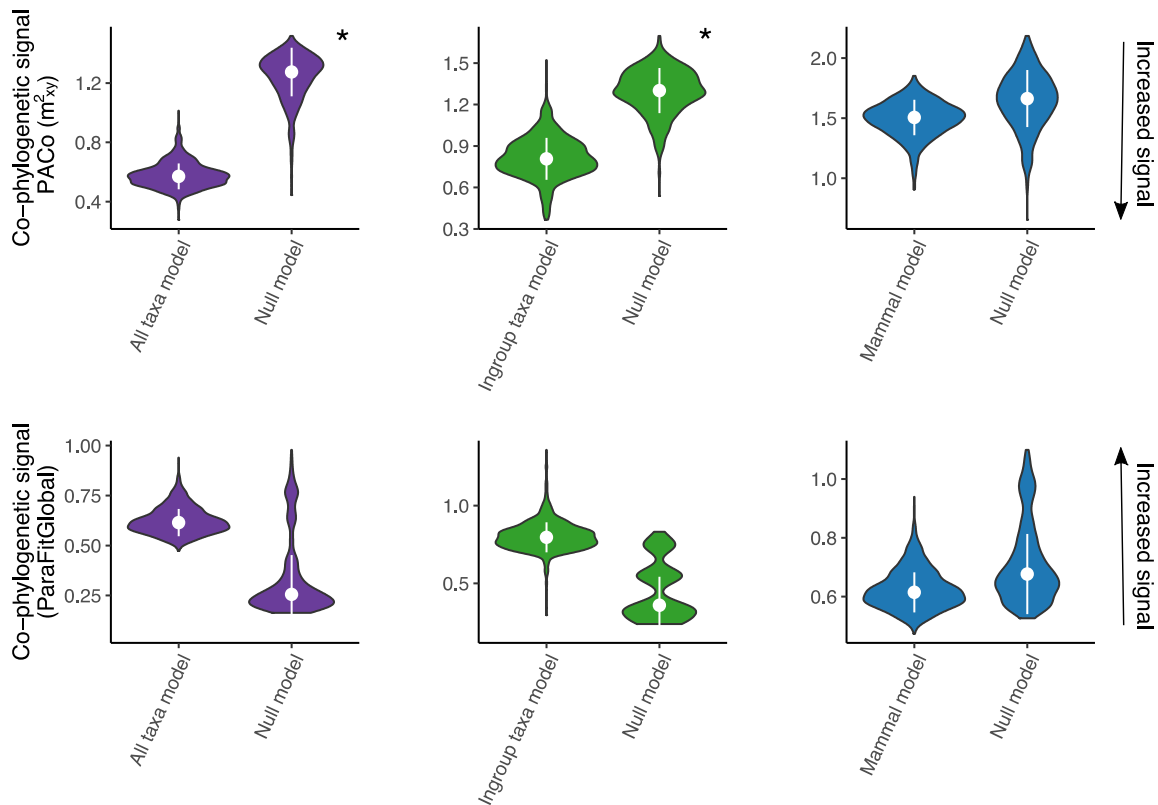
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806

807 **Fig. S3. Characterization of DrDV delta antigen proteins.** (A) Alignment of delta antigen
808 protein sequences for mammalian, snake and avian deltaviruses. Shading indicates level of
809 similarity across all sequences, with regions of highest identity in black. (B) Putative sequence of
810 the large DAg for the DrDV-B virus from the site CAJ1. The RNA editing site is marked with a
811 black arrow; UAG has been edited to UGG yielding a tryptophan residue (W).

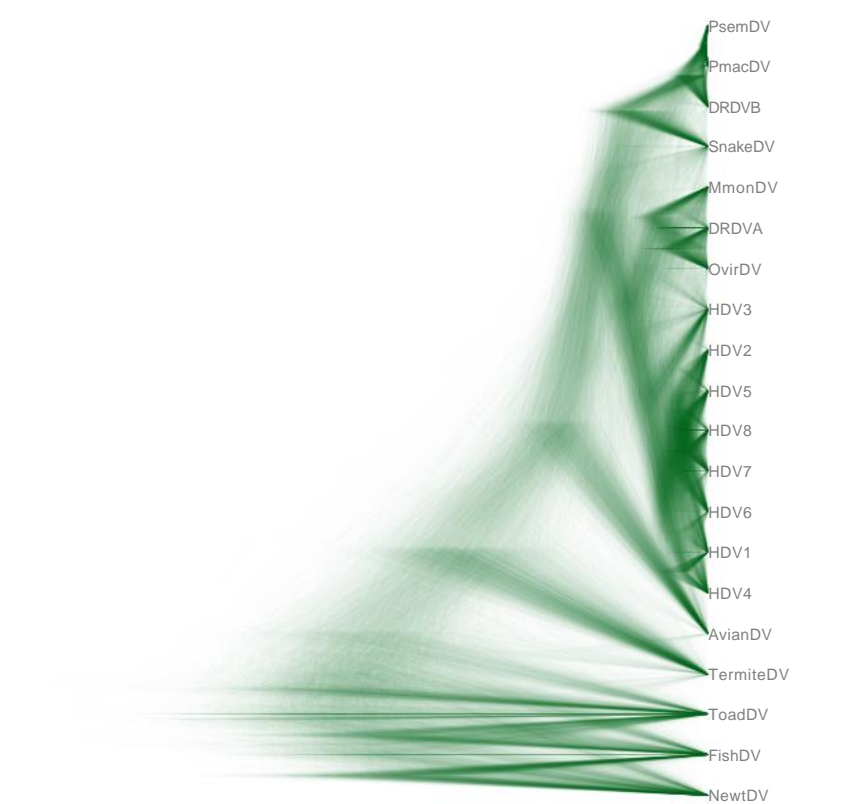
812



813 **Fig. S4. Co-phylogenetic signal in subsets of the deltavirus phylogeny.** Violin plots show the
814 degree of dependence of 1,000 phylogenies from the posterior of the StarBeast analysis (Fig. S5)
815 on the host phylogeny relative to null models, with the median and standard deviation. Data subsets
816 are colored as in Fig. 2B (All taxa: purple+green+blue, Ingroup: green+blue, mammals: blue)
817 Distributions are shown for analyses performed using PACo (top row) and ParaFit (bottom row).
818 Asterisks show significant dependence of the virus phylogeny on the host phylogeny ($P < 0.05$).
819 Note that lower values of the empirical model relative to the null model represent increased signal
820 of co-phylogeny in PACo while higher values represent increased signal of co-phylogeny in
821 ParaFit.

822

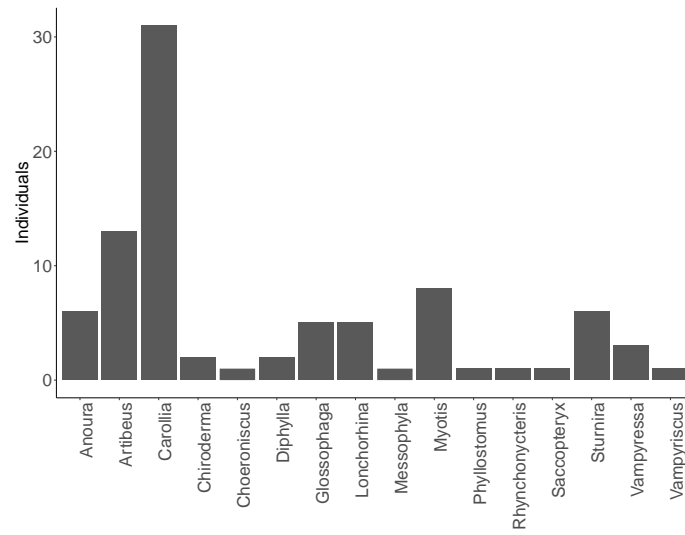
823



824

825 **Fig. S5. Uncertainty of deep relationships in the deltavirus phylogeny.** The DensiTree shows
826 the distribution of 1,000 posterior trees from the StarBeast analysis, highlighting uncertainties in
827 the evolutionary relationships among divergent deltavirus-like taxa.

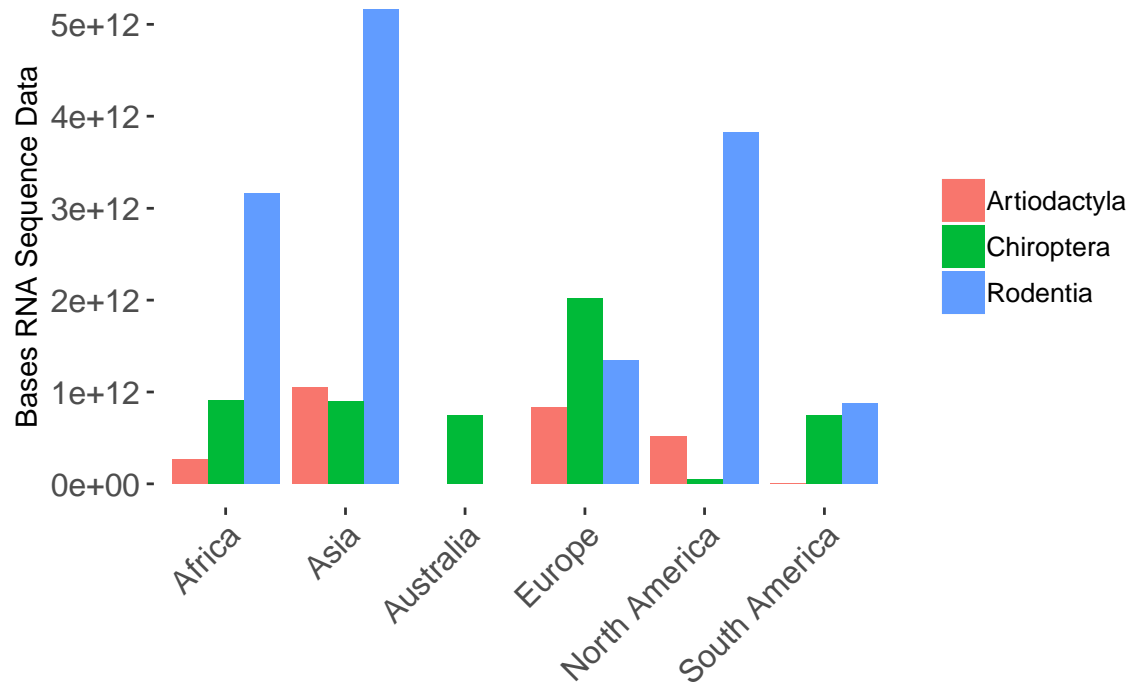
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829

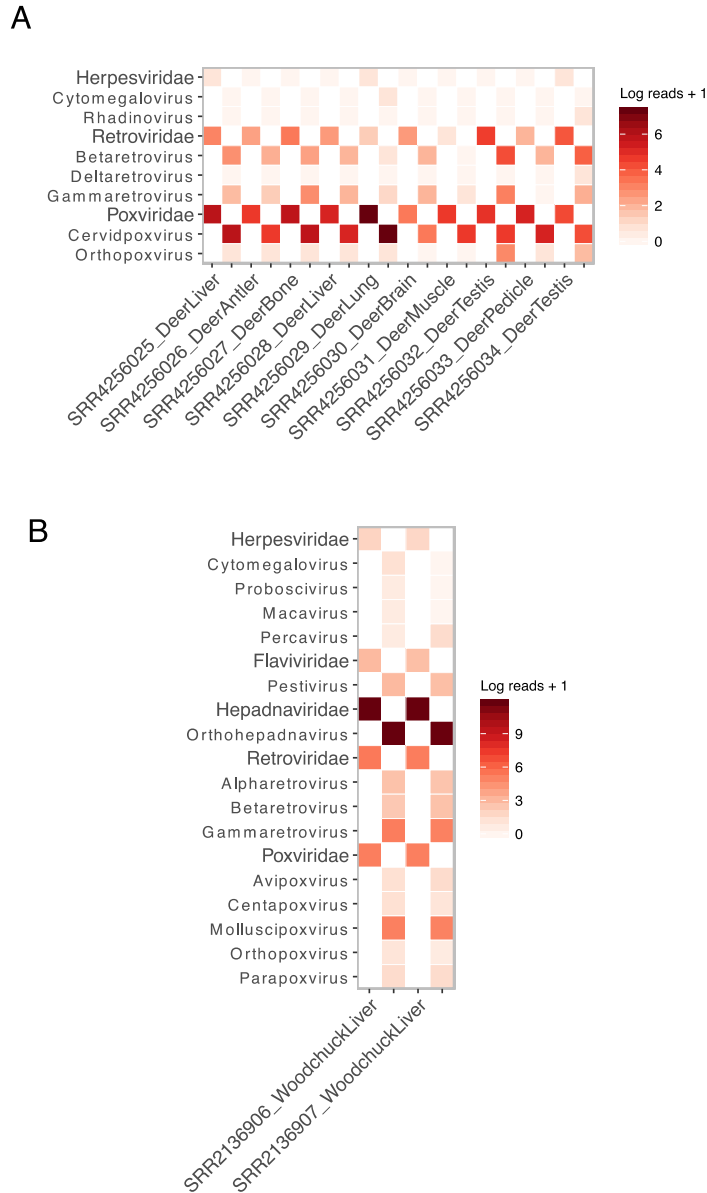
830 **Fig. S6. Counts of non-*D. rotundus* bat species saliva swabs individually screened by RT-**
831 **PCR for DrDV-B. Bars group bats by genus.**

832



833

834 **Fig S7. Continent level geographic biases in RNA sequence data examined by Serratus.** Bars
835 are colored by mammalian order; data shown are limited to the three orders in which deltaviruses
836 were detected.



837

838 **Fig S8. Candidate helper viruses for the OvirDV and MmonDV datasets.** Mammal-infecting
 839 viral communities are shown for (A) *O. virginianus* libraries sequenced by RNASeq from (17),
 840 several of which contained OvirDV and (B) two *M. monax* samples infected with MmonDV from
 841 (18). Viral families (in larger font) and genera are shown in adjacent columns for each sample,
 842 with families on the left and genera on the right.

843

844

845 **Supplementary Tables**

846 **Table S1. Pooled bat saliva samples from Peru analyzed by metagenomic sequencing.**

Genus	Species	Individuals in pool	Raw reads	Deltavirus contig length (bp)
<i>Carollia</i>	<i>perspicillata</i>	10*	28,700,978	N
<i>Glossophaga</i>	<i>soricina</i>	5	24,079,752	N
<i>Desmodus</i>	<i>rotundus</i>	10	28,946,275	921†
<i>Diphylla</i>	<i>ecaudata</i>	2	25,023,095	N
<i>Anoura</i>	<i>geoffroyi</i>	6	18,569,505	N
	<i>peruana</i>			
<i>Artibeus</i>	<i>lituratus</i>	10	14,966,399	N
	<i>obscurus</i>			
	<i>planirostris</i>			
	<i>fraterculus</i>			
<i>Myotis</i>	<i>oxyotus</i>	8	19,934,479	N
	<i>unidentified sp</i>			
<i>Sturnira</i>	<i>erythromos</i>	6	11,348,995	N
	<i>unidentified sp</i>			
<i>Vampyressa/</i>	<i>bidens</i>	4	13,734,389	N
<i>Vampyriscus</i>	<i>unidentified sp</i>			
<i>Rare species</i>	<i>Chiroderma trinitatum</i>	8	16,746,795	N
	<i>Chiroderma salvini</i>			
	<i>Choeroniscus minor</i>			
	<i>Rhynchonycteris naso</i>			
	<i>Saccopteryx bilineata</i>			
	<i>Messophyla macconelli</i>			
	<i>Phyllostomus discolor</i>			
	<i>Rhinophylla pumilio</i>			

847

848 *Pool included individual CP-1 in which DrDV-B was detected by RT-PCR

849 †Pool was identical to CAJ1 pool where DrDV was initially discovered, confirming the ability to detect

850 deltaviruses when they are known to be present

851 **Table S2. Deltavirus positive cohorts evaluated by mapping reads from related libraries to**
 852 **novel deltavirus genomes.**

SRA accession	Pool ID	Hosts _§	DV Reads _¶
ERR2756783	AAC_H_F	<i>Desmodus rotundus</i>	2 (DrDV-A)
ERR2756784	AAC_H_SV*	<i>Desmodus rotundus</i>	189 (DrDV-A) 18 (DrDV-B)
ERR2756785	AAC_L_F	<i>Desmodus rotundus</i>	0
ERR2756786	AAC_L_SV	<i>Desmodus rotundus</i>	0
ERR2756787	AMA_L_F_NR	<i>Desmodus rotundus</i>	0
ERR2756788	AMA_L_F_R	<i>Desmodus rotundus</i>	0
ERR2756789	AMA_L_SV	<i>Desmodus rotundus</i>	0
ERR2756790	CAJ_L_F_NR	<i>Desmodus rotundus</i>	0
ERR2756791	CAJ_L_F_R	<i>Desmodus rotundus</i>	0
ERR2756792	CAJ_L_SV	<i>Desmodus rotundus</i>	4
ERR2756793	CAJ_H_F_1	<i>Desmodus rotundus</i>	0
ERR2756794	CAJ_H_F_2	<i>Desmodus rotundus</i>	4
ERR2756795	CAJ_H_SV*	<i>Desmodus rotundus</i>	169
ERR2756796	HUA_H_F	<i>Desmodus rotundus</i>	2
ERR2756797	HUA_H_SV	<i>Desmodus rotundus</i>	0
ERR2756798	LMA_L_F_NR	<i>Desmodus rotundus</i>	0
ERR2756799	LMA_L_F_R	<i>Desmodus rotundus</i>	0
ERR2756800	LMA_L_SV_NR	<i>Desmodus rotundus</i>	45
ERR2756801	LMA_L_SV_R*	<i>Desmodus rotundus</i>	320
ERR2756802	LR_L_F_NR	<i>Desmodus rotundus</i>	0
ERR2756803	LR_L_F_R	<i>Desmodus rotundus</i>	0
ERR2756804	LR_L_SV	<i>Desmodus rotundus</i>	0
ERR3569452	AMA2_H	<i>Desmodus rotundus</i>	0
ERR3569453	AMA2_SV	<i>Desmodus rotundus</i>	0
ERR3569454	API1_H	<i>Desmodus rotundus</i>	0
ERR3569455	API1_SV	<i>Desmodus rotundus</i>	0
ERR3569456	API17_H	<i>Desmodus rotundus</i>	0
ERR3569457	API17_SV	<i>Desmodus rotundus</i>	2 (DrDV-A)
ERR3569458	API140_H	<i>Desmodus rotundus</i>	32

ERR3569459	API140_SV	<i>Desmodus rotundus</i>	0
ERR3569460	API141_H	<i>Desmodus rotundus</i>	0
ERR3569461	API141_SV	<i>Desmodus rotundus</i>	0
ERR3569462	AYA1_H	<i>Desmodus rotundus</i>	2
ERR3569463	AYA1_SV	<i>Desmodus rotundus</i>	0
ERR3569464	AYA7_H	<i>Desmodus rotundus</i>	0
ERR3569465	AYA7_SV	<i>Desmodus rotundus</i>	18
ERR3569466	AYA11_H	<i>Desmodus rotundus</i>	0
ERR3569467	AYA11_SV*	<i>Desmodus rotundus</i>	591
ERR3569468	AYA12_H	<i>Desmodus rotundus</i>	0
ERR3569469	AYA12_SV	<i>Desmodus rotundus</i>	2
ERR3569470	AYA14_H	<i>Desmodus rotundus</i>	0
ERR3569471	AYA14_SV*	<i>Desmodus rotundus</i>	228 (DrDV-A) 2 (DrDV-B)
ERR3569472	AYA15_H	<i>Desmodus rotundus</i>	0
ERR3569473	AYA15_SV	<i>Desmodus rotundus</i>	0
ERR3569474	CAJ1_H	<i>Desmodus rotundus</i>	0
ERR3569475	CAJ1_SV*	<i>Desmodus rotundus</i>	172
ERR3569476	CAJ2_H	<i>Desmodus rotundus</i>	4
ERR3569477	CAJ2_SV†	<i>Desmodus rotundus</i>	0
ERR3569478	CAJ4_H	<i>Desmodus rotundus</i>	0
ERR3569479	CAJ4_SV†	<i>Desmodus rotundus</i>	0
ERR3569480	CUS8_H	<i>Desmodus rotundus</i>	0
ERR3569481	CUS8_SV	<i>Desmodus rotundus</i>	4
ERR3569482	HUA1_H	<i>Desmodus rotundus</i>	0
ERR3569483	HUA1_SV	<i>Desmodus rotundus</i>	0
ERR3569484	HUA2_H	<i>Desmodus rotundus</i>	5
ERR3569485	HUA2_SV	<i>Desmodus rotundus</i>	0
ERR3569486	HUA3_H	<i>Desmodus rotundus</i>	0
ERR3569487	HUA3_SV	<i>Desmodus rotundus</i>	0
ERR3569488	HUA4_H	<i>Desmodus rotundus</i>	0
ERR3569489	HUA4_SV	<i>Desmodus rotundus</i>	0
ERR3569490	LMA5_H	<i>Desmodus rotundus</i>	0
ERR3569491	LMA5_SV	<i>Desmodus rotundus</i>	8

ERR3569492	LMA6_H	<i>Desmodus rotundus</i>	0
ERR3569493	LMA6_SV*	<i>Desmodus rotundus</i>	173
ERR3569494	LR2_H	<i>Desmodus rotundus</i>	0
ERR3569495	LR2_SV	<i>Desmodus rotundus</i>	0
ERR3569496	LR3_H	<i>Desmodus rotundus</i>	0
ERR3569497	LR3_SV	<i>Desmodus rotundus</i>	0
SRR7910142	Pm_03	<i>Peropteryx macrotis</i>	0
SRR7910143	Pm_01*	<i>Peropteryx macrotis</i>	346
SRR7910144	Pm_02	<i>Peropteryx macrotis</i>	2
SRR7910145	NI_02‡	<i>Nyctinomops laticaudatus</i>	0
SRR7910146	NI_03‡	<i>Nyctinomops laticaudatus</i>	0
SRR7910147	Mk_01‡	<i>Myotis keaysi</i>	0
SRR7910148	Mk_02‡	<i>Myotis keaysi</i>	0
SRR7910149	Mm_02‡	<i>Mormoops megalophylla</i>	0
SRR7910150	Mm_03‡	<i>Mormoops megalophylla</i>	0
SRR7910151	Aj_03‡	<i>Artibeus jamaicensis</i>	0
SRR7910152	Mm_01‡	<i>Mormoops megalophylla</i>	0
SRR7910153	Aj_01‡	<i>Artibeus jamaicensis</i>	0
SRR7910154	Aj_02‡	<i>Artibeus jamaicensis</i>	0
SRR7910155	Mk_03‡	<i>Myotis keaysi</i>	0
SRR7910156	NI_01‡	<i>Nyctinomops laticaudatus</i>	0
SRR4256025	Deer-Liver-1-male	<i>Odocoileus virginianus</i>	0
SRR4256026	Deer-Antler-2-male	<i>Odocoileus virginianus</i>	12
SRR4256027	Deer-Bone-1-male	<i>Odocoileus virginianus</i>	0
SRR4256028	Deer-Liver-2-male	<i>Odocoileus virginianus</i>	0
SRR4256029	Deer-Lung-1-male	<i>Odocoileus virginianus</i>	0
SRR4256030	Deer-Brain-2-male	<i>Odocoileus virginianus</i>	12
SRR4256031	Deer-Muscle-2-male	<i>Odocoileus virginianus</i>	14
SRR4256032	Deer-Testis-1-male	<i>Odocoileus virginianus</i>	0
SRR4256033	Deer-Pedicle-male*	<i>Odocoileus virginianus</i>	9265
SRR4256034	Deer-Testis-2-male	<i>Odocoileus virginianus</i>	7

853

854 * Pools in which full deltavirus genomes were detected

855 † Pools in which DrDV was detected in the saliva of one or more individuals in the pool by RT-PCR, but
856 were negative for deltavirus detection through metagenomics

857 ‡ Species in which deltaviruses were not detected but which came from the same study

858 § *D. rotundus* samples represent saliva (SV) and fecal (F/H) samples pooled across multiple individuals
859 from different sites. Samples from other Neotropical bats (*P. macrotis*, *N. laticaudatus*, *M. keaysi*, *M.*
860 *megalophylla*, *A. jamaicensis*) represent liver samples from unique individuals. *O. virginianus* samples
861 represent different tissues pooled across multiple individuals. Read mapping of samples from different
862 individuals and time points to the MmonDV genome is described in (1)

863 ¶ Samples from *D. rotundus* were mapped to DrDV-A and DrDV-B genomes. All *D. rotundus* read counts
864 refer to DrDV-B genomes unless specifically noted as DrDV-A. In the case of libraries with matches to
865 both, the number of reads mapping is broken down by DrDV-genome. Samples from *P. macrotis*, *N.*
866 *laticaudatus*, *M. keaysi*, *M. megalophylla*, *A. jamaicensis* were mapped to the PmacDV genome. Samples
867 from *O. virginianus* were mapped to OvirDV.

868

869 **Table S3. Summary statistics for bat deltavirus genomes and protein domain homology**
870 **analysis of predicted DrDV small delta antigens from saliva metagenomic pools.**

871

Site	Lineage	Genome (nt)	GC content (%)	Intramolecular base pairing (%)	Delta antigen (aa)	Hhpred top hit	Probability top hit	e-value	Identity top hit (%)	Genbank accession
AYA14	DrDV-A	1694	55	73.8	194	Oligomerization	99.86	2.8e-25	59	MT649207
AYA11	DrDV-B	1692	54.3	75.3	196	domain of	99.86	5.60E-25	45	MT649206
CAJ1	DrDV-B	1692	53.8	74.3	196	hepatitis delta	99.86	5.40E-25	45	MT649208
LMA6	DrDV-B	1694	54.3	74.6	196	antigen	99.85	8.30E-25	45	MT649209

872

873

874 **Table S4. Primers used to screen samples for DrDV by RT-PCR and HBV by PCR.**

875

Primer	PCR Round	Sequence (5'-3')
DrDV-A		
DrDV_F1_GenoA	1&2	AGGGGTCTTTTTGGGAAATT
DrDV_R1_GenoA	1	AAGAAGAAGCAACTATCCGG
DrDV_R2_GenoA	2	CATCCAAGAGACCAAGAGAG
DrDV-B		
DrDV_F1_GenoB	1	TTCCCTTG YTGCTCCAGTTG
DrDV_R1_GenoB	1	CGGTAAGAAGAAACCTCCAA
DrDV_F2_GenoB	2	CCAGTTGTTTCTTCTTGTTCTC
DrDV_R2_GenoB	2	AAAAAGAAAGAGAGAACTGGAAAAA
DrDV Delta Antigen		
DeltaAntigenF1_GenoB	1	TCTGGTCTTATCTTTCTTACCTTAT
DeltaAntigenR1_GenoB	1	AAACCTTCCTTTATTCTATTTCGAA
DeltaAntigenR1_GenoA	1	CCTTTACCTTTAATTCTCTTG GTAA
DeltaAntigenF1_GenoA	1	GCCTCGAATAATAAGAAGAAAATTT
HBV Primers*		
HBV-F248	1&2	CTAGATTBGTGGTGGACTTCTCTCA
HBV-R397	2	GATARAACGCCGCAGATACATCCA
HBV-R450a	1	TCCAGGAGAACCAAYAAGAAAGTGA
HBV-R450b	1	TCCAGGAGAACCAAYAAGAAAGTGA

876

877 *Primer sequences and PCR protocol described in (38)

878

879

880

881 **Table S5. Colony level demographic characteristics and PCR-based screening results of**
 882 **vampire bat blood and saliva for DrDV and HBV.**

883

Colony	Prop Male*	Prop Adult†	DrDV-A		DrDV-B		HBV	
			Saliva	Blood	Saliva	Blood	Saliva	Blood
AYA1	0.6	1	0/20	0/20	3/20	0	0/3	0/20
AYA11	0.6	0.95	0/20	0/20	2/20	0	0/3	0/20
AYA14	0.4	0.65	1/20	0/20	4/20	0	0/8	0/20
AYA15	0.55	0.75	0/20	0	0/20	0	0	0
CAJ1	0.75	0.9	0/20	0	5/20	0/20	0/10	0/20
CAJ2	0.55	0.95	0/20	0	6/20	6/20	0/6	0/20
CAJ3	0.7	1	0/20	0	4/20	0	0/4	0
CAJ4	0.35	0.75	0/20	0	2/20	0	0/2	0
LMA4	0.65	0.75	0/20	0	0/20	0	0/1	0
LMA5	0.65	0.9	0/20	0	5/20	0	0/5	0
LMA6	0.35	1	0/20	0	7/20	4/20	0/9	0/19
LMA12	0.5	0.9	0/20	0	3/20	0	0/3	0
Total	-	-	1/240	0/60	41/240	10/60	0/54	0/119

884

885 *Proportion of males at each colony (alternative is females)

886 †Proportion of adults at each colony (alternatives are juveniles or subadults)

887

888 **Table S6. Test of association between DrDV-B phylogeny and sample location at the**
 889 **regional (department) and colony level.**

890

Level	Index	Observed value (95% CI)	Null value (95% CI)	p-value
Region	AI*	0.22 (0-0.58)	2.46 (1.95-2.94)	0
	PS†	4 (3-5)	17.73 (15.41-19.35)	0
	MC‡ (LMA)	9.29 (5-14)	1.97 (1.41-2.98)	0.001
	MC‡ (CAJ)	11.24 (9-19)	2.81 (2.12-3.94)	0.001
	MC‡ (AYA)	2.67 (1-5)	1.26 (1-1.96)	0.02
Colony	AI*	2.23 (1.69-2.76)	3.63 (3.21-3.92)	0
	PS†	19.19 (18-20)	29.23 (27.45-30.81)	0
	MC‡ (LMA6)	4.98 (5-5)	1.18 (1-1.94)	0.001
	MC‡ (LMA5)	2.52 (1-3)	1.13 (1-1.43)	0.002
	MC‡ (CAJ2)	3.21 (2-6)	1.57 (1.14-2.39)	0.01
	MC‡ (CAJ1)	1 (1-1)	1.13 (1-1.53)	1
	MC‡ (AYA1)	1.09 (1-2)	1.01 (1-1.05)	1
	MC‡ (CAJ3)	1.01 (1-1)	1.07 (1-1.32)	1
	MC‡ (AYA11)	1.03 (1-1)	1.01 (1-1.05)	1
	MC‡ (AYA14)	1.05 (1-2)	1.04 (1-1.15)	1
	MC‡ (CAJ4)	1.16 (1-2)	1.01 (1-1.05)	1
	MC‡ (LMA12)	1 (1-1)	1.04 (1-1.15)	1

891

892 *Association Index

893 † Parsimony Score

894 ‡ Monophyletic Clade size

895

896 **Supplementary References**

897

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