Paramyxoviruses in Old World fruit bats (Pteropodidae): an open database and synthesis of sampling effort, viral positivity, and coevolution

Short title: Paramyxovirus sampling and prevalence in pteropodid bats

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Note: This study involves no new data collection or live participants and therefore does not require an Ethics Statement.

Abstract

Over the past 25 years, research interest in the links between Old World fruit bats (family Pteropodidae) and paramyxoviruses has driven a rapid proliferation of surveillance studies in this system. We performed a systematic review and data synthesis of all published paramyxovirus studies of wild pteropodids. Here, we present an open, static, PRISMA-compliant database called pteroparamyxo, which includes 1,482 records of prevalence or seroprevalence from 119 studies published between 1971 and 2023. Using this dataset, we examined biases in sampling effort, predictors of viral positivity, tissue tropism, and pteropodid–paramyxovirus coevolution. We found significant spatial and taxonomic bias in sampling effort, largely driven by overrepresentation of *Pteropus*–henipavirus systems; in particular, African bats were undersampled for paramyxoviruses relative to Asian and Oceanian taxa. On the viral side, henipaviruses (specifically Hendra, Nipah, and Cedar viruses) were overrepresented relative to pararubulaviruses. We also identified substantial non-random variability in paramyxovirus prevalence and seroprevalence. Spleens and pooled fecal samples from pteropodids yielded the highest PCR positivity, while samples from Oceania tended to have slightly higher seroprevalence than those from Asia and Africa. Collection year was not a significant predictor of positivity, suggesting limited overall change in paramyxovirus PCR prevalence or seroprevalence over the last several decades. Finally, we found weak evidence of pteropodid–paramyxovirus coevolution, supporting relatively frequent host-switching within this system. Our study highlights critical sampling gaps to address in future surveillance studies and provides preliminary evidence for sample and tissue types to prioritize in field- and museum-based sampling.

Author Summary

Paramyxoviruses are a family of viruses that include the human measles and mumps viruses as well as emerging zoonoses like Hendra and Nipah henipaviruses. These henipaviruses spill over from wild fruit bats to domestic animals and humans in Australia and Asia, causing high fatality rates with no known human treatments or vaccines. The occurrence and prevalence of paramyxoviruses across the entire fruit bat family (Pteropodidae) has never been formally investigated, as published detection attempts have not yet been consolidated into a database. We conducted a systematic review of the existing literature on pteropodid–paramyxovirus sampling efforts and compiled an open database for researchers to reference and use. We then leveraged this novel database for a suite of analyses and demonstrated that Australian and Asian fruit bats are disproportionately sampled for paramyxoviruses while African species are understudied, despite the fact that African samples have slightly higher rates of paramyxovirus detection. We identified sample types that may be particularly likely to yield viral detections, a finding which can help guide future survey efforts using field studies as well as museum collections. We also demonstrate that paramyxoviruses appear liable to switch host species within Pteropodidae, challenging earlier hypotheses that these viruses are host specific.

Introduction

Anthropogenic change is driving increased viral transmission across species boundaries, including spillover of zoonotic viruses from wildlife to humans (1). One particular viral family of interest is the *Paramyxoviridae*, negative-strand RNA viruses identified across vertebrate groups. Paramyxoviruses cause particularly high disease burdens in mammals and birds, with varying degrees of host specificity (2). For example, the subfamily *Orthoparamyxovirinae* contains the genera *Morbillivirus* (e.g., host-specific measles virus and broadly infecting canine

74 distemper virus) and *Henipavirus* (e.g., zoonotic bat-borne Hendra and Nipah viruses, which
75 cause fatal encephalitis in horses and humans) (3). The subfamily *Rubulavirinae* contains the
76 genera *Orthorubulavirus* (e.g., mumps and human parainfluenza viruses) and *Pararubulavirus*
77 (e.g., Tioman virus, Menangle virus, and other recently isolated bat-borne viruses) (3). To
78 understand the host range, geographic range, and potential spillover risks of paramyxoviruses, a
79 more comprehensive picture of host–paramyxovirus relationships is critical.

80 Bats are known to host a variety of paramyxoviruses and have been suggested as the
81 ancestral hosts of *Paramyxoviridae* (4). In particular, Old World fruit bats (family Pteropodidae)
82 have been implicated as reservoir hosts of two highly pathogenic henipaviruses and other
83 potentially zoonotic paramyxoviruses. This ecologically and morphologically diverse bat family
84 of roughly 200 species is widespread across Asia, Oceania, and Africa (5). Immunity-related
85 genes in pteropodids have evolved at higher rates relative to other chiropteran families, and some
86 pteropodid-specific mutations are associated with decreased inflammatory responses to
87 pathogens (6). A balance of viral tolerance and immune defense strategies may explain these fruit
88 bats' apparent ability to host viruses that are highly pathogenic in other mammals (7).
89 Henipaviruses, including Hendra virus (8), Nipah virus (9), Ghanaian bat henipavirus (10), and
90 Cedar virus (11), have been detected in pteropodids. Novel pararubulaviruses have also been
91 isolated from pteropodids (12–15). Some studies have noted apparent clustering of related
92 paramyxoviruses in certain pteropodid species, raising the possibility of host specificity and
93 host-virus coevolution (16), though this has not been quantitatively tested. This growing body of
94 evidence linking pteropodids to paramyxoviruses has also prompted further research on the
95 ecological links between these bats and their viruses (17–20), which have implications for
96 downstream spillover risk (21,22).

97 Pteropodids are largely frugivorous and nectarivorous, and loss of native habitat has
98 driven many of these species to seek food sources in urban areas (e.g., public and private
99 gardens), increasing the likelihood of human–bat interactions (5). Climate and land-use change
100 have been linked to the spillover of henipaviruses from pteropodids (22,23), and the geographic
101 (and possibly host) ranges of paramyxoviruses appear to be expanding in certain cases—for
102 example, the recent Nipah virus spillovers and associated fatalities in South India (24,25). The
103 resulting interest in henipaviruses may have created a bias toward studying *Pteropus* bats in
104 Australia and South and Southeast Asia (23). Sampling disparities such as these, coupled with a
105 lack of consolidated data, prevent a deeper understanding of global changes in viral occurrence,
106 distributions, and spillover rates over time (26). Large, taxonomically broad host–virus
107 association databases partially address this issue, but these presence-only databases lack negative
108 results by nature and also often lack fine-grained metadata. While taxonomically specific
109 databases exist for other viral families like coronaviruses (27), interactions between pteropodids
110 and the broader *Paramyxoviridae* have not been comprehensively compiled, despite the fact that
111 these bats have been heavily sampled for henipaviruses and other paramyxoviruses (28–30). The
112 absence of such a synthesis limits the identification of patterns in viral prevalence, which is
113 critical for spillover prediction and mitigation (31).
114 To address these gaps and enable formal synthesis of pteropodid–paramyxovirus
115 associations, we built a standardized, PRISMA-compliant database of all published detection
116 attempts of paramyxoviruses in wild pteropodid bats, including metadata on viral taxonomy, host
117 taxonomy, sampling and detection methodologies, seasonality, location, and date, as well as
118 including data on negative results. We then used the resulting dataset to perform a series of
119 phylogenetic comparative analyses and formal meta-analyses to better understand pteropodid–

120 paramyxovirus relationships and the current state of the associated literature. To this end, we
121 investigated: (1) geographic and taxonomic (both host and viral) sampling gaps in this system;
122 (2) key predictors of paramyxovirus prevalence and seroprevalence in pteropodids, including
123 potential tissue tropism; and (3) the degree of pteropodid–paramyxovirus coevolution or host-
124 switching.

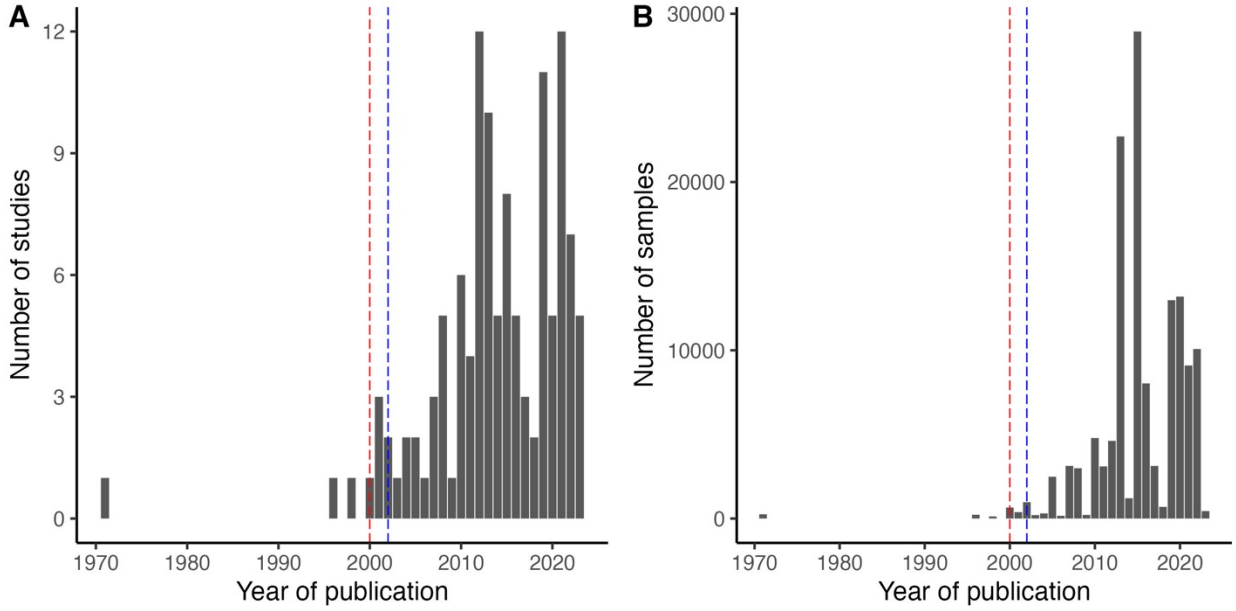
125 **Results**

126 *Overview of dataset*

127 Our final database consists of 1,482 individual records from 119 studies of
128 paramyxoviruses in pteropodids published between 1971 and 2023 (Supplementary Data 1).
129 Each record constitutes a detection attempt associated with a unique combination of the
130 following columns: host identification (at the finest taxonomic resolution available, typically
131 species), virus identification (at the finest taxonomic resolution available, typically genus or
132 species), detection type (single or pooled), study type (cross-sectional, longitudinal, or both),
133 general detection method (PCR, serology, or viral isolation), specific detection method (i.e., type
134 of PCR or serological assay), gene or antigen target, sample type (e.g., tissue, swab, blood,
135 urine), type of tissue tested, country, state/region, site (with coordinates if reported), sampling
136 year, and sampling month/season. Each record also includes the number of samples tested, the
137 number of positive samples, and the reported prevalence.

138 Only three of the 119 studies were published before the year 2000, reflecting the rapid
139 increase in interest in pteropodid–henipavirus relationships after the discovery of Hendra and
140 Nipah virus in the 1990s (Fig. 1). Of the total records, 66% involve PCR detection attempts, 27%
141 involve serological detection attempts, and 7% involve viral isolation attempts. Over 67% of
142 records focus on the viral subfamily *Orthoparamyxovirinae*, and 66% of records involve

143 henipaviruses specifically (the remaining 1% reflected "henipa-like" orthoparamyxoviruses).
144 Another 11% involve the viral subfamily *Rubulavirinae*, with the remaining records attempting
145 to detect any paramyxovirus (typically with PCR primers targeting the L gene). Detections of
146 unclassified sequences comprise under 5% of all records. Fine-grained spatial studies of
147 paramyxovirus prevalence are limited by available spatial metadata; only 21% of records have
148 associated latitude and longitude listed in the original studies. However, 68% include a specific
149 site (or multiple sites), 89% include an administrative area within a country (e.g., state, province,
150 or district), and 99% name a specific country, allowing for broader studies of geographic biases
151 in sampling effort (and potential georeferencing for future studies). The most common sample
152 types in our dataset are blood (29%), urine (27%), swabs (22%), and tissue (16%). Detailed
153 breakdowns of sampling effort and viral positivity by tissue type, detection method, and viral
154 subfamily are included in Supplementary Tables 1 and 2.



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156 **Fig. 1. Sampling effort of paramyxoviruses in Pteropodidae across time.** (A) Studies by year
157 of publication. (B) Samples by year of publication (as collection year is often reported as a range

of time). The vertical dashed red and blue lines indicate the first isolations of Hendra and Nipah virus, respectively, from *Pteropus* bats (8,9).

Spatial sampling biases

Our dataset includes studies in all three regions within the Pteropodidae range (Africa, Asia, and Oceania) across 33 countries/territories (Fig. 2; Supplementary Table 3). However, sampling effort has been highly heterogeneous across countries, with the number of studies per country ranging from one to 29 (Fig. 2a) and the number of samples per country ranging from 21 (Gabon) to 58,590 (Australia) (Fig. 2b). In particular, large geographic sampling gaps are found in central and southern Africa (Fig. 2). A generalized linear model (GLM) of binary sampling effort and subsequent post-hoc comparisons did not reveal any statistically significant variation across the three regions ($\chi^2 = 4.66$, $p = 0.10$, $R^2 = 0.03$) (Table 1a). However, the intensity of sampling did vary across regions, in terms of both the number of studies per country ($\chi^2 = 26.23$, $p < 0.0001$, $R^2 = 0.11$) and the number of samples per country ($\chi^2 = 147{,}816$, $p < 0.0001$, $R^2 = 0.38$). Specifically, there have been more pteropodid–paramyxovirus studies and samples from Oceania than from Asia or Africa (Table 1b,c), a difference likely driven by an ongoing research focus on the *Pteropus*–Hendra virus system in Australia. There have also been more samples collected from Asia than from Africa (Table 1c).

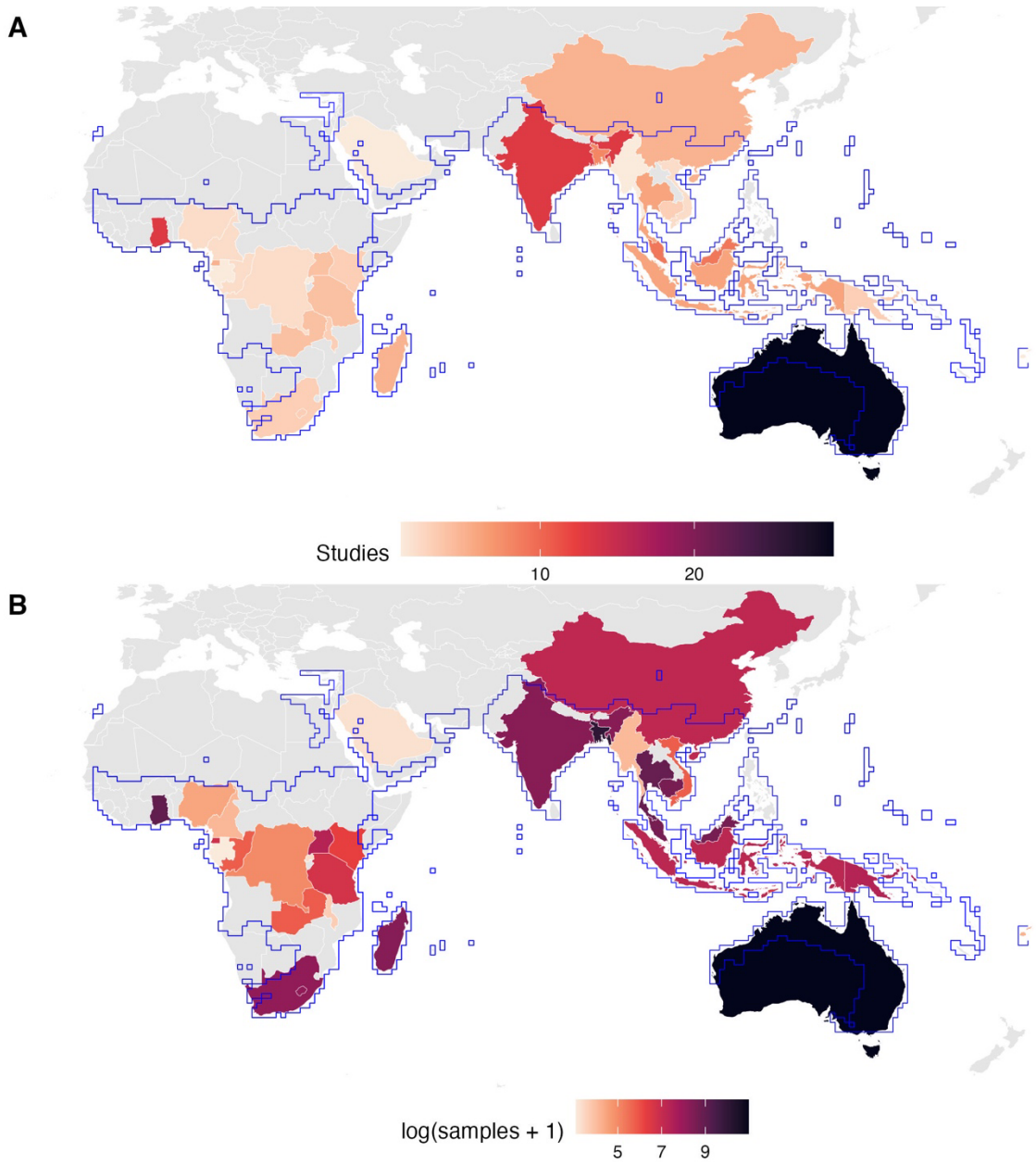

Fig. 2. Geographic distribution of paramyxovirus sampling effort in pteropodids. The aggregated Pteropodidae IUCN distribution is outlined in blue. Note that in the UN Geoscheme, Saudi Arabia and Indonesia are classified as Asia. (A) Number of studies per country (ranging from one to 29). (B) Number of samples tested per country (ranging from 21 to 58,590, presented on a log scale).

Table 1. Post-hoc pairwise comparisons from GLMs of sampling effort by region. (A) binary sampling; (B) number of studies; and (C) number of samples across countries as a function of general geographic region. The *p*-values are adjusted for multiple comparisons using a Bonferroni correction. Confidence intervals are back-transformed from the log scale.

Comparison	Odds ratio (95% confidence interval)	z ratio	*p* value
A. Binary sampling effort (binomial response)			
Africa / Asia	1.39 (0.49-3.94)	0.76	1.00
Africa / Oceania	3.71 (0.74-18.54)	1.95	0.15
Asia / Oceania	2.67 (0.51-14.02)	1.42	0.47
B. Number of studies (Poisson response)			
Africa / Asia	0.69 (0.44-1.07)	-2.02	0.13
Africa / Oceania	0.30 (0.18-0.51)	-5.51	**<0.0001**
Asia / Oceania	0.44 (0.26-0.74)	-3.77	**0.0005**
C. Number of samples (Poisson response)			
Africa / Asia	0.29 (0.28-0.29)	-151.60	**<0.0001**
Africa / Oceania	0.06 (0.06-0.06)	-355.39	**<0.0001**
Asia / Oceania	0.20 (0.20-0.21)	-261.79	**<0.0001**

Taxonomic sampling biases

Pteropodids sampled for paramyxoviruses include 58 of the 194 species and 23 of the 43 genera in the family. Using a phylogeny of all the Pteropodidae (32), we found intermediate phylogenetic signal in binary sampling effort ($D = 0.85$), departing from both phylogenetic randomness ($p = 0.03$) and Brownian motion models of evolution ($p = 0$). We then applied phylogenetic factorization, a graph-partitioning algorithm (33), which identified one clade with a disproportionately high fraction of sampled species, including *Rousettus amplexicaudatus*, *R. spinalatus*, *R. leschenaultii*, *R. obliviosus*, *R. madagascariensis*, *R. aegyptiacus*, *R. lanosus*, and *R. celebensis*, as well as the genera *Epomops*, *Epomophorus*, *Micropteropus*, *Nanonycteris*, *Hypsignathus*, *Megaloglossus*, and *Myonycteris* (Fig. 3a, Table 2a). Among sampled species, the number of studies per species range from one to 24 and was randomly distributed across the host

203 phylogeny (Pagel's $\lambda = 0.00$), departing from Brownian motion clustering ($p < 0.0001$) but not
204 from phylogenetic randomness ($p = 1$). *Rousettus leschenaultii*, *R. obliviosus*, *R.*
205 *madagascariensis*, and *R. aegyptiacus* are overrepresented relative to other taxa, as have been
206 many species of *Pteropus*; however, *P. chrysoproctus*, *P. tonganus*, *P. neohibernicus*, and *P.*
207 *temminckii* have been significantly understudied (Fig. 3b, Table 2b). The number of samples per
208 species range from one to 25,047 and showed moderate phylogenetic signal ($\lambda = 0.59$), departing
209 from both Brownian motion clustering ($p < 0.0001$) and phylogenetic randomness ($p = 0.01$).
210 The single identified oversampled clade constitutes 16 *Pteropus* species, including those heavily
211 sampled for Hendra and Nipah viruses (e.g., *P. giganteus*, *P. lylei*, *P. poliocephalus*, *P. alecto*)
212 (Fig. 3c, Table 2c).

213 On the viral side, 16 of the 78 recognized species in the family *Paramyxoviridae* (3) have
214 been detected in Pteropodidae, comprising three genera: *Henipavirus*, *Orthorubulavirus*, and
215 *Pararubulavirus*. We found strong, highly conserved phylogenetic signal in binary sampling
216 effort ($D = -0.90$), departing from phylogenetic randomness ($p = 0$) but not from Brownian
217 motion models of evolution ($p = 0.99$). Phylogenetic factorization did not identify any viral
218 clades differing significantly in the fraction of detected species (Fig. 3d, Table 2d). Among
219 detected viruses, the number of studies per virus range from one to 55 and displayed strong
220 phylogenetic signal ($\lambda = 0.93$), departing from phylogenetic randomness ($p = 0.04$) but not from
221 Brownian motion ($p = 0.60$). Hendra, Nipah, and Cedar henipaviruses have been overstudied,
222 while Tioman, Teviot, and Menangle pararubulaviruses have been understudied (Fig. 3e, Table
223 2e). The number of samples per virus range from nine to 52,006 samples and also show strong
224 phylogenetic signal ($\lambda = 1.00$), departing from phylogenetic randomness ($p = 0.04$) but not from
225 Brownian motion ($p = 1.00$). Again, Hendra (52,006 samples), Nipah (46,089 samples), and

226 Cedar (2,139 samples) henipaviruses are overrepresented relative to all other detected
227 paramyxoviruses (Fig. 3f, Table 2f).
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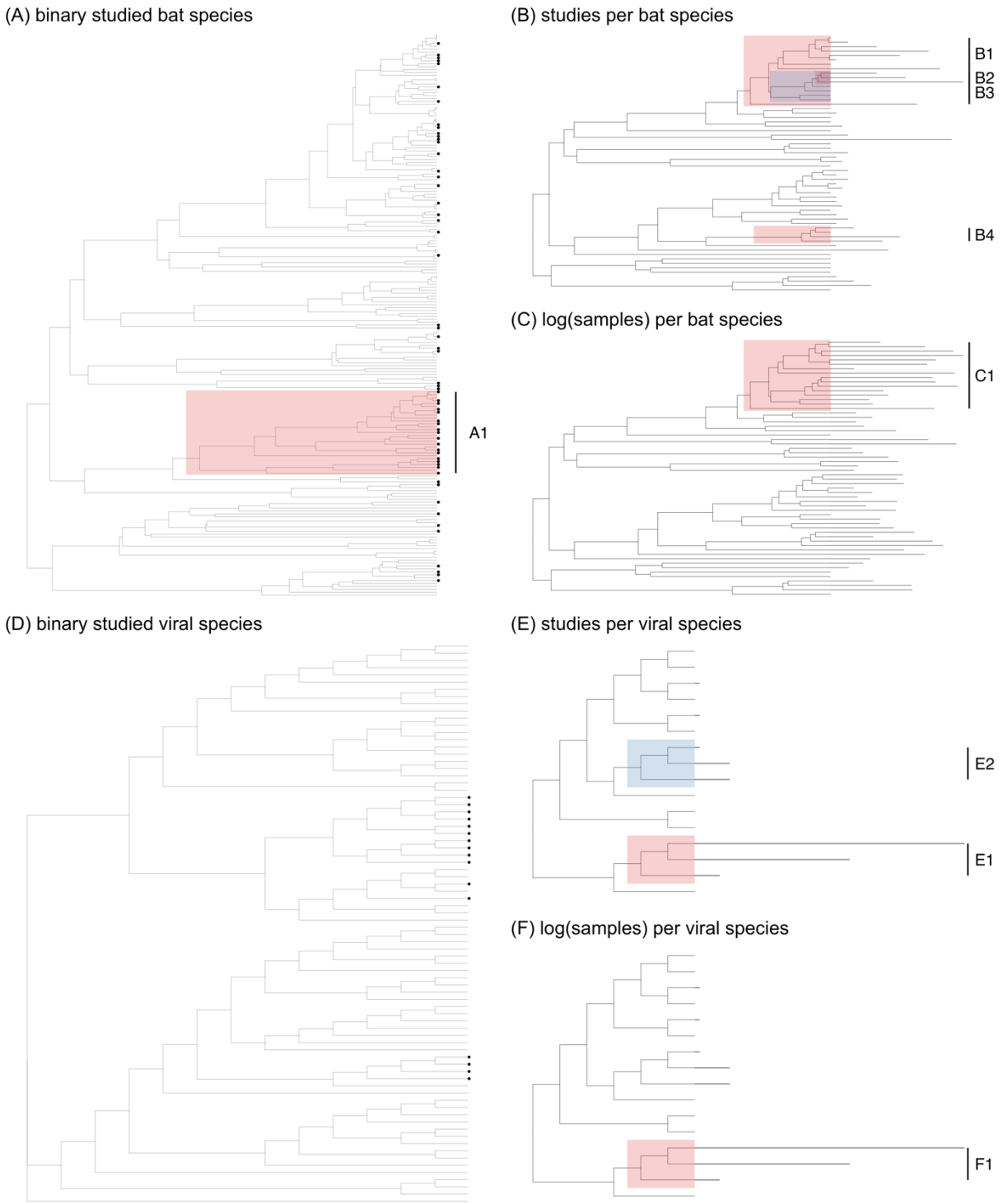

Fig. 3. Host and viral taxonomic biases in pteropodid–paramyxovirus sampling effort. (A) Whether or not a bat species has been sampled for paramyxoviruses, across all Pteropodidae (black dots indicate species that have been sampled); (B) number of studies per sampled bat

species; (C) logged number of samples per sampled bat species; (D) whether or not a viral species has been detected in Pteropodidae (black dots indicate viruses that have been detected); (E) number of studies screening for each detected viral species; (F) logged number of samples screened for each detected viral species. Red and blue shading indicates clades with greater or lesser sampling effort, respectively, in comparison to all other taxa (as identified by phylogenetic factorization), with clade numbers corresponding to Table 2. In plots (B), (C), (E), and (F), segment length represents relative sampling effort.

268 **Table 2. Bat and virus clades differing significantly from the rest of their respective trees in**
269 **terms of different measures of sampling effort, as identified by phylogenetic factorization.**
270 (A) Whether or not a bat species has been sampled for paramyxoviruses, across all Pteropodidae
271 (0: not sampled; 1: sampled); (B) number of studies per sampled bat species; (C) logged number
272 of samples per sampled bat species; (D) whether or not a viral species has been detected in
273 Pteropodidae (no significant clades identified); (E) number of studies screening for each detected
274 viral species; (F) logged number of samples screened for each detected viral species (Fig. 3). We
275 report all significant clades detected through phylogenetic factorization (identified with Holm's
276 sequentially rejective test at a family-wise error rate of 5%), the taxa and number of species
277 within those clades, and the mean sampling effort for the clade compared to the rest of the tree.
278 Bat taxonomy follows Upham et al. 2019 and viral taxonomy follows Rima et al. 2019.

Clade	Taxa	Number of species	Clade mean	Other mean
A) Binary sampling of Pteropodidae				
A1	*Rousettus amplexicaudatus*, *Rousettus spinalatus*, *Rousettus leschenaultii*, *Rousettus obliviosus*, *Rousettus madagascariensis*, *Rousettus aegyptiacus*, *Epomops*, *Epomophorus*, *Micropteropus*, *Nanonycteris*, *Hypsignathus*, *Megaloglossus*, *Myonycteris*, *Rousettus lanosus*, *Rousettus celebensis*	29	0.62	0.24
B) Number of studies of sampled pteropodids				
B1	*Pteropus chrysoproctus*, *Pteropus scapulatus*, *Pteropus vampyrus*, *Pteropus giganteus*, *Pteropus lylei*, *Pteropus seychellensis*, *Pteropus rufus*, *Pteropus poliocephalus*, *Pteropus hypomelanus*, *Pteropus conspicillatus*, *Pteropus alecto*, *Pteropus tonganus*, *Pteropus neohibernicus*, *Pteropus melanotus*, *Pteropus griseus*, *Pteropus temminckii*	16	8.44	3.38
B2	*Pteropus hypomelanus*, *Pteropus conspicillatus*, *Pteropus alecto*	3	15.67	4.18
B3	*Pteropus chrysoproctus*, *Pteropus tonganus*, *Pteropus neohibernicus*, *Pteropus temminckii*	4	1.00	5.06
B4	*Rousettus leschenaultii*, *Rousettus obliviosus*, *Rousettus madagascariensis*, *Rousettus aegyptiacus*	4	7.25	4.59
C) Logged number of samples of sampled pteropodids				
C1	*Pteropus chrysoproctus, Pteropus scapulatus, Pteropus vampyrus, Pteropus giganteus, Pteropus lylei, Pteropus seychellensis, Pteropus rufus, Pteropus poliocephalus, Pteropus hypomelanus, Pteropus conspicillatus, Pteropus alecto, Pteropus tonganus, Pteropus neohibernicus, Pteropus melanotus, Pteropus griseus, Pteropus temminckii*	16	6.59	3.91
D) Binary detection of paramyxoviruses in Pteropodidae				
E) Number of studies of detected paramyxoviruses				
E1	*Hendra henipavirus*, *Nipah henipavirus*, *Cedar henipavirus*	3	31	2.31
E2	*Tioman pararubulavirus*, *Teviot pararubulavirus*, *Menangle pararubulavirus*	3	6	8.08
F) Logged number of samples of detected paramyxoviruses				
F1	*Hendra henipavirus*, *Nipah henipavirus*, *Cedar henipavirus*	3	9.76	5.82

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Heterogeneity and predictors of paramyxovirus positivity

We fitted two initial, intercept-only phylogenetic meta-analysis models (one for PCR prevalence and one for seroprevalence) that included random effects of within- and between-study variation, host genus, genus-level phylogeny, and country (32,34). We observed significant heterogeneity among positivity estimates in both the PCR dataset ($I^2 = 87.8\%$, $Q = 3{,}793.41$, $p < 0.0001$) and the serological dataset ($I^2 = 96.5\%$, $Q = 9{,}770.71$, $p < 0.0001$). This heterogeneity was mostly explained by between-study (65.0% PCR; 40.0% serology) and within-study (14.1% PCR; 26.6% serology) effects. Bat phylogeny explained 0% of the heterogeneity in the PCR dataset but 28.1% in the serological dataset, and other genus effects accounted for 0% of the heterogeneity in both datasets. Country effects accounted for 8.7% and 1.8% of the heterogeneity in the PCR and serological datasets, respectively. None of the fixed effects included in our models were significantly associated with paramyxovirus prevalence or seroprevalence in Pteropodidae, with all confidence intervals crossing zero and no significant post-hoc pairwise comparisons; this may be due to the limited sample size of these separate datasets (particularly serology; $n = 313$). However, in the PCR model, serum, feces, tissue, and urine appeared to have slightly higher prevalence than the intercept (blood), and African and Asian samples had slightly higher prevalence than the intercept (Oceania). Cross-sectional studies and longitudinal studies appear to yield similar PCR prevalence rates. In the serology model, African and Asian samples had slightly lower prevalence than samples from Oceania. In both models, Eidolinae, Pteropodinae, and Rousettinae subfamilies had slightly higher viral detection than the intercept (Cynopterinae), as did Macroglossusinae in the PCR model (Fig. 4).

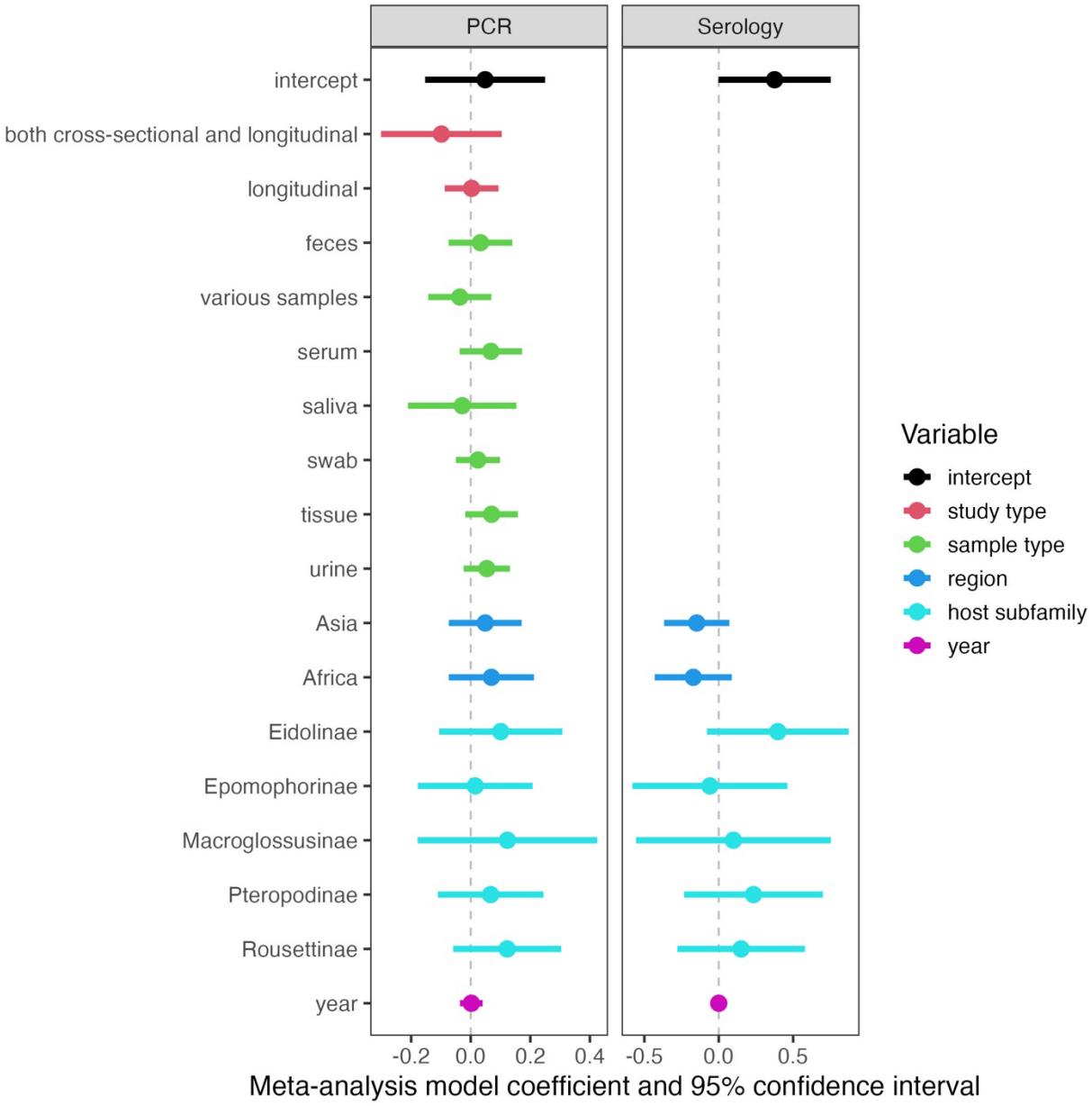

Fig. 4. Phylogenetic meta-analysis model coefficients and 95% confidence intervals, estimated with REML for each dataset. Colours indicate the variables included in each model. Transparent confidence intervals indicate non-significant effects that cross zero (the vertical dashed line). The intercept contains the following reference levels: cross-sectional (study type); blood (sample type); region (Oceania); and Cynopterinae (host subfamily). Sample sizes are 786 PCR prevalence estimates and 313 seroprevalence estimates.

Because tissue type had to be excluded from the phylogenetic meta-analysis models due to collinearity with other predictors, we separately investigated paramyxovirus tissue tropism in Pteropodidae by calculating PCR prevalence for each tissue type. We found that PCR prevalence was highest in spleen samples (6.9%), followed by pooled tissue samples from multiple organs (6.5%), pooled fecal samples (6.5%), pooled swabs (5.4%), and pooled urine samples (4.2%) (Fig. 5). All other tissue or sample types yielded around or under 2% prevalence.

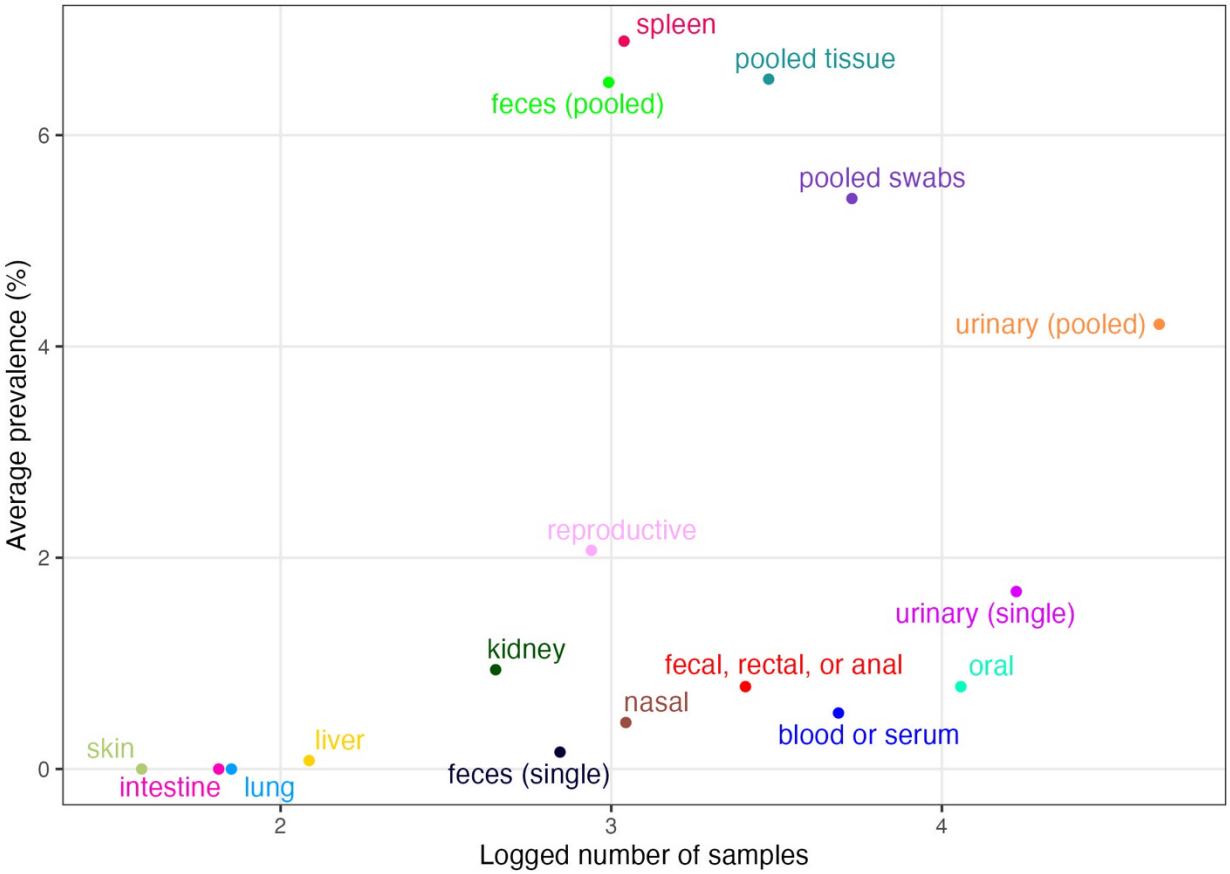

Fig. 5. PCR prevalence of paramyxoviruses in different pteropodid tissues. Plotted by relative sampling effort (represented by the log base 10 of the number of samples screened per tissue type).

322 *Cophylogenetic analysis*

323 Lastly, we tested the dependence of the viral phylogeny on the host phylogeny using the
324 Procrustean Approach to Cophylogeny (PACo) (35). This matrix included 23 known PCR or
325 viral isolation links between 12 pteropodid species and 13 ICTV-ratified paramyxovirus species
326 (Fig. 6). Only three viral species (*Hendra henipavirus*, *Nipah henipavirus*, and *Tioman*
327 *pararubulavirus*) have been identified in more than one bat species, while over half of the
328 included bat species (7/12) have links to more than one virus (Fig. 6). When compared to 5,000
329 randomly permuted host–virus matrices, we found no statistically significant congruence
330 between the two phylogenies in our dataset (m^2_{XY} = 9,827.11, p = 0.69). Failure to reject the null
331 hypothesis indicates a high degree of host-switching by paramyxoviruses within Pteropodidae.
332 However, the links between *Pteropus* species and henipaviruses and some pararubulaviruses
333 (Teviot, Tioman, Menangle) had lower residuals and therefore demonstrated more support for
334 host–virus coevolution, as did the links between *Rousettus leschenaultii* and Tuhoko
335 pararubulaviruses (Fig. 6, Supplementary Table 4).
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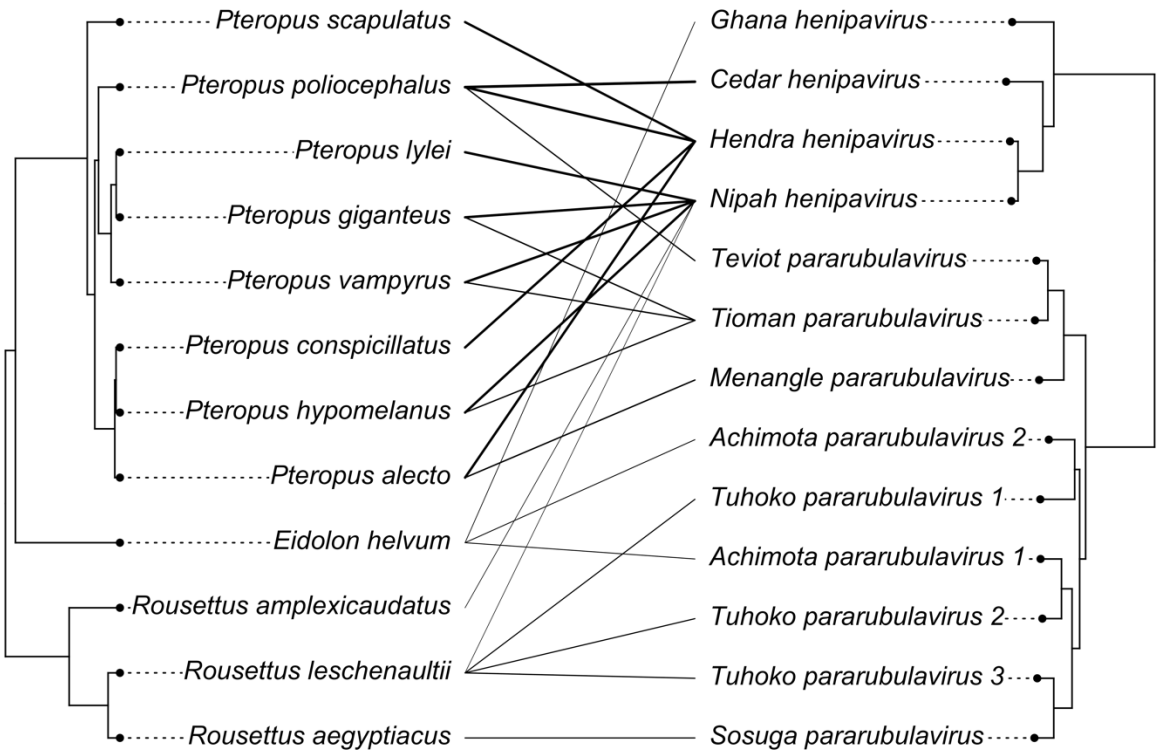

Fig. 6. Tanglegram indicating known links (PCR detections or viral isolations) between pteropodid species and ICTV-recognized paramyxovirus species. Darker links signify smaller residuals from PACo (suggesting more support for coevolution), while lighter links signify higher residuals (more support for host-switching) (Supplementary Table 4). The phylogeny on the left indicates evolutionary relationships among pteropodid species in this dataset, with *Eidolon helvum* and the *Pteropus* genus forming a sister clade to *Rousettus* species. The phylogeny on the right indicates evolutionary relationships among viral species in this dataset, with henipaviruses and pararubulaviruses forming two distinct clades.

Discussion

The isolation of Hendra and Nipah viruses from flying foxes in the early 2000s sparked widespread research interest in the relationship between pteropodid bats and paramyxoviruses

350 (8,9). We sought to aggregate all published records of pteropodid–paramyxovirus associations
351 and prevalence into a PRISMA-compliant, open, static database (Supplementary Fig. 1). Our
352 database, named pteroparamyxo, includes 1,482 records of viral detection attempts from 119
353 studies published between 1971 and 2023, which allows for formal synthesis of sampling and
354 positivity across space, time, and host/viral taxonomy. This consolidation is critical for
355 addressing questions about changes in viral dynamics (26). Our focus on gathering detailed
356 metadata for a viral and host family-specific database will enable robust downstream models to
357 be trained on this taxonomic subset, an approach with higher predictive capacity than broader
358 models (36).

359 Spatial and taxonomic analyses of this dataset confirm suspected trends and biases in
360 sampling effort. Pteropodids in Australia and, to a lesser degree, South Asia (particularly India
361 and Bangladesh) have been heavily sampled for paramyxoviruses, in terms of both number of
362 studies and number of samples collected (Fig. 2). These trends are likely driven by studies of
363 Hendra and Nipah virus spillover events and high numbers of under-roost urine samples
364 collected following outbreaks (37,38). Much of Africa and Asia remains understudied for
365 paramyxoviruses in fruit bats, despite evidence of henipavirus spillover into human populations
366 in Cameroon, for example (39), a country which remains undersampled in our dataset (Fig. 2).
367 This sampling gap is also notable in light of the fact that African samples had slightly higher
368 PCR prevalence than samples from Oceania, and some African bat subfamilies (Eidolinae and
369 Rousettinae) had slightly higher PCR prevalence and seroprevalence than other subfamilies (Fig.
370 4). Taken together, this suggests missed opportunities for additional detection of
371 paramyxoviruses in African pteropodids (Fig. 4).

372 Similarly, taxonomic sampling biases abound on both the host and viral side. In terms of
373 binary sampling effort, a clade containing some *Rousettus* species and closely related genera
374 (*Epomops*, *Epomophorus*, *Micropteropus*, *Nanonycteris*, *Hypsignathus*, *Megaloglossus*,
375 *Myonycteris*) has a disproportionately high fraction of species sampled for paramyxoviruses.
376 Among sampled taxa, some species in the highly studied genera *Pteropus*, *Rousettus*, and
377 *Eidolon* are significantly overrepresented relative to other species; however, certain *Pteropus*
378 species have been understudied (e.g., largely island-dwelling species like *P. chrysoproctus*, *P.*
379 *tonganus*, *P. neohibernicus*, and *P. temminckii*) (Fig. 3; Table 2). The occurrence and prevalence
380 of paramyxoviruses in these bat species are currently unknown due to very small sample sizes,
381 and future surveillance studies could target these taxa accordingly. Those that are particularly
382 elusive or difficult to sample in the wild may be good candidates for museum-based viral
383 screening, an approach which allows for efficient and taxonomically broad sampling across
384 space and time (40). Further, while the scope of this study was limited to Pteropodidae,
385 collecting and consolidating viral data from non-pteropodid bats could allow for broader
386 analyses of bat–paramyxovirus coevolution. Preliminary evidence suggests that some
387 insectivorous bat families host paramyxovirus clades distinct from those in pteropodid bats, a
388 finding that could be more robustly assessed with additional sampling and explicit
389 cophylogenetic tests (4,41).
390 On the viral side, the majority of studies have focused on henipaviruses; consequently,
391 Hendra, Nipah, and Cedar viruses are overrepresented, while pararubulaviruses are
392 comparatively neglected in the literature (Fig. 3; Table 2). Outside of the viruses detected in
393 pteropodids, there is a vast diversity of other mammalian and vertebrate paramyxoviruses that do
394 not appear in our dataset (Fig. 3d), including paramyxoviruses identified in insectivorous bats

395 (42–44). Conversely, 70 records in our database involve unclassified paramyxovirus sequences
396 that could appear anywhere in the viral tree. Viral taxonomy can be unstable and often lags
397 behind host taxonomy (3); future ICTV updates may recognize additional bat-borne
398 paramyxovirus species. Still, based on published links between 13 recognized paramyxovirus
399 species and 12 named pteropodid species (other isolated viruses were generally attributed to
400 *Pteropus* spp.), we performed a cophylogenetic analysis that revealed little evidence of
401 phylogenetic congruence between hosts and viruses, contrary to tentative hypotheses of
402 pteropodid–paramyxovirus coevolution posed in earlier surveillance studies (16). The strongest
403 coevolutionary signal was found in *Pteropus–Henipavirus* systems, though this result was not
404 statistically significant and may be an artifact caused by heavy sampling of *Pteropus* species for
405 henipaviruses. There was more support for host-switching among other links (e.g.,
406 *Rousettus*/*Eidolon*–pararubulaviruses). These findings raise the possibility of paramyxovirus
407 expansion into new pteropodid hosts and geographic areas, which may have implications for
408 spillover into human and domestic animal populations.
409 Our results also hint at methodologies and sample types that might yield higher
410 paramyxovirus prevalence. In general, urine, tissue, serum, and fecal samples appeared to have
411 higher PCR prevalence than blood, whereas saliva and swab samples had lower prevalence (Fig.
412 4). A more in-depth look at tissue tropism suggested that spleen and pooled feces are
413 undersampled relative to their typical prevalence, as these tissues have the highest PCR
414 prevalence among specified sources (i.e., excluding pooled tissue) (Fig. 5; Supplementary Table
415 1). These sample types could be good priorities for future surveillance studies of
416 paramyxoviruses in pteropodids and offer specific options for both invasive (e.g., spleen
417 extraction during necropsy) and non-invasive (e.g., under-roost fecal collection (37)) sampling

418 approaches. Both cross-sectional and longitudinal studies yielded similar PCR prevalence (Fig.
419 4), suggesting that both are suitable approaches for surveillance studies and that the study type
420 can be selected based on the research question at hand.

421 Here, we have: (1) highlighted the major spatial and taxonomic sampling gaps in the
422 pteropodid–paramyxovirus system; (2) identified sample types, regions, host subfamilies, and
423 study types associated with higher prevalence or seroprevalence; and (3) found evidence of host-
424 switching by paramyxoviruses within Pteropodidae. Importantly, we found no evidence that
425 paramyxovirus prevalence or seroprevalence in pteropodids has changed in recent decades, as
426 collection year was not a significant predictor in our models (Fig. 4). However, syntheses and
427 meta-analyses like this one are limited in scope and statistical robustness by data gaps as well as
428 a lack of stratified and standardized metadata reported in existing studies. Where possible,
429 researchers should report the specific tissue types, localities, sampling dates, taxonomic
430 identifications, and other metadata included in pooled samples, and provide detection estimates
431 at as fine-grained a level as possible to enable more detailed analysis of pteropodid–
432 paramyxovirus associations. This will help inform sampling protocols for surveillance of wild
433 bats and museum specimens. Our published dataset constitutes a first step toward gathering,
434 standardizing, and stratifying surveillance data to guide future studies of paramyxoviruses, which
435 continue to pose threats to human health. Addressing the sampling gaps outlined here will be
436 critical in developing a more complete picture of these bat–virus systems.
437
438
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440

441 **Methods**

442 *Systematic review*

443 To generate our database of paramyxovirus detection attempts in wild pteropodids, we
444 followed the PRISMA protocol (Supplementary Fig. 1) (45). On August 3rd, 2023, we
445 systematically searched PubMed and Web of Science with the following string: (pteropodid* OR
446 pteropid* OR "fruit bat" OR "fruit bats" OR "flying fox" OR "flying foxes" OR *Acerodon* OR
447 *Aethalops* OR *Alionycteris* OR *Aproteles* OR *Balionycteris* OR *Boneia* OR *Casinycteris* OR
448 *Chironax* OR *Cynopterus* OR *Desmalopex* OR *Dobsonia* OR *Dyacopterus* OR *Eidolon* OR
449 *Eonycteris* OR *Epomophorus* OR *Epomops* OR *Haplonycteris* OR *Harpyionycteris* OR
450 *Hypsignathus* OR *Latidens* OR *Macroglossus* OR *Megaerops* OR *Megaloglossus* OR
451 *Melonycteris* OR *Mirimiri* OR *Myonycteris* OR *Nanonycteris* OR *Neopteryx* OR *Nesonycteris*
452 OR *Notopteris* OR *Nyctimene* OR *Otopteropus* OR *Paranyctimene* OR *Penthetor* OR
453 *Pilonycteris* OR *Plerotes* OR *Ptenochirus* OR *Pteralopex* OR *Pteropus* OR *Rousettus* OR
454 *Scotonycteris* OR *Sphaerias* OR *Stenonycteris* OR *Styloctenium* OR *Syconycteris* OR
455 *Thoopterus*) AND (paramyxovirus* OR *Paramyxoviridae* OR henipavirus* OR Hendra OR
456 Nipah OR rubulavirus* OR morbillivirus*). We identified 1,260 records in total, and proceeded
457 to screen 863 titles and abstracts from non-duplicated records. Studies were excluded if they did
458 not include new (i.e., not republished) data on paramyxovirus detection attempts in wild bats—
459 therefore, any studies of captive bats or experimental studies of bat-derived cell lines were
460 excluded. We identified 137 articles for further full-text screening, of which 119 met the
461 inclusion criteria for our study.

462 *Data collection*

463 Our final database included 119 studies and 1,482 records. Where prevalence was not
464 reported in the original studies, it was calculated from the number of positive and tested samples
465 before meta-analysis. For viral isolation attempts where positivity rate or number of positive
466 samples was not reported, we included a binary variable (0: unsuccessful detection; 1: successful
467 detection). Therefore, the same bat may be included in multiple records, if the study tested
468 multiple samples (e.g., different tissue types or detection methods) per individual bat. For papers
469 where over 100 sampling events are detailed, sampling events were summarized by location,
470 species, and tissue type. When original studies did not separate samples by species, locations,
471 tissue types, or years, all information was included in one record (separated by commas in the
472 relevant cells). Before downstream analyses, these pooled records were filtered out when
473 relevant, but they remain in the database for researchers who may be interested in specific
474 species, tissue types, or sites. When original studies did not report all the metadata for a study
475 (e.g., sampling season), these were not imputed.

476 *Spatial and taxonomic sampling biases*

477 Our dataset included bat samples from 33 countries in three geographical regions (Africa,
478 Asia, and Oceania; countries classified according to the United Nations Geoscheme). All
479 analyses were conducted in R (version 4.2.1) (46). We first visualized the number of studies and
480 (log-scale) samples per country by building maps with the aggregated Pteropodidae IUCN range
481 map overlaid for context. We then fitted three GLMs to these country-level variables with region
482 as the categorical predictor and one of three respective response variables: binary sampling status
483 (whether or not a country had been sampled for paramyxoviruses in pteropodids) as a binomial
484 response, with all countries in these three regions included; the number of unique studies per
485 country as a Poisson-distributed response, with only sampled countries included; and the number

486 of samples per country as another Poisson-distributed response, with only sampled countries
487 included. We assessed the fit of GLMs using McFadden's R^2 ('performance' package) and made
488 individual region-to-region pairwise comparisons using the 'emmeans' package (47,48).
489 To assess taxonomic sampling biases, we first aligned all sampled bats (n = 58) and
490 viruses (n = 16) identified at the species level to host (32) and virus (3) phylogenies. To do so,
491 we relabeled species names to fit the published taxonomy where necessary (e.g., *Pteropus*
492 *medius* was relabeled as *P. giganteus* for the purpose of taxonomic backbone alignment, though
493 we retain the currently recognized names in our published database). For all bat and virus species
494 in the phylogenies, we created a binary response variable representing whether or not each
495 species had been sampled or sampled for, respectively. For those species present in our dataset,
496 we calculated the number of unique studies and total samples associated with each bat and virus
497 species. To assess the degree of phylogenetic signal in sampling effort across bats and viruses,
498 we used the 'caper' package (49) to calculate D (<0: highly conserved; 0: phylogenetic clustering
499 under Brownian motion; 0-1: intermediate phylogenetic signal; 1: phylogenetically random
500 sampling distribution; >1: overdispersed) for binary sampling effort and Pagel's lambda (λ) (0:
501 phylogenetically random sampling distribution; 0-1: intermediate phylogenetic signal; 1:
502 phylogenetic clustering under Brownian motion) for the $\log_{10}$-transformed number of studies and
503 samples. Finally, we used the package 'phylofactor' (Washburne et al. 2017) to apply
504 phylogenetic factorization, a graph-partitioning algorithm, to both the bat and virus phylogenies.
505 This approach partitioned binary sampling effort (binomial response), number of studies
506 (Poisson distribution), and number of samples (Gaussian distribution due to an extreme skew in
507 number of samples and consequent transformation via natural log) across the trees using iterative
508 GLMs, producing a list of bat or virus clades (containing at least three species) that differ

509 significantly from the rest of the tree in sampling effort. We used Holm's sequentially rejective
510 test with a family-wise error rate of 5% to account for multiple comparisons and determine the
511 number of statistically significant clades (50).

512 *Meta-analysis of viral and antibody positivity*

513 We used the 'metafor' package (34) to run two hierarchical meta-analysis models for two
514 separate viral positivity datasets: PCR detection (n = 786 records) and serological detection (n =
515 313 records). The response variables in our models were the Freeman-Tukey double arcsine-
516 transformed proportions of paramyxovirus PCR-positive or seropositive samples and their
517 sampling variances. Models were fitted using restricted maximum likelihood (REML) and
518 included bat genus, genus-level phylogeny (32), and country as random effects along with an
519 observation-level random effect nested inside a study-level random effect; all models were also
520 weighted by inverse sampling variance. We used genus instead of species as a random effect in
521 these models to retain records from bats not identified to the species level (e.g., unspecified
522 Australian *Pteropus* spp.). We calculated the contribution of heterogeneity (true variability rather
523 than noise) to variance in viral positivity (I^2) for the entire dataset, as well as the proportional
524 contribution for each random effect. Our models included all available non-collinear predictors.
525 For the PCR model, we included fixed effects of region (Oceania, Asia, Africa), study type (cross
526 sectional, longitudinal, or both), sample type (feces, serum, saliva, swab, tissue, various),
527 sampling year, and host subfamily (Cynopterinae, Eidolinae, Epomophorinae, Macroglossusinae,
528 Pteropodinae, Rousettinae); for the serology model, we included fixed effects of region (Oceania,
529 Asia, Africa), sampling year, and host subfamily (Cynopterinae, Eidolinae, Epomophorinae,
530 Macroglossusinae, Pteropodinae, Rousettinae). For records reflecting detection attempts across a

range of sampling years, the midpoint of the start and end years was used to calculate the average "sampling year".

To assess paramyxovirus prevalence in different tissue types (as tissue type was a collinear predictor in all possible models and therefore could not be included), we also calculated overall viral prevalence per tissue type for records using PCR.

Cophylogenetic analysis

We lastly used PACo, with the 'paco' package, to assess coevolution between paramyxoviruses and pteropodids and thus the dependence of the viral phylogeny on the host phylogeny (35). For this analysis, we defined a known association as either a PCR detection or viral isolation of an ICTV-recognized paramyxovirus species in a described species of pteropodid, thereby excluding serological detections (due to lower viral specificity) and any PCR or viral culture detection in bats recognized only to the genus level (e.g., unspecified Australian *Pteropus* spp.). We conducted a goodness-of-fit test to evaluate how well a codivergence model fits the matrix of known host–virus links, and then established its statistical significance by fitting the model to 5000 randomizations of the matrix and testing the null hypothesis that host and virus species are randomly associated. We generated residuals for each host–virus link (smaller residuals indicate more support for coevolution, and higher residuals indicate more support for host-switching). We then visualized the host–virus links with a tanglegram of all known links in our dataset using the package 'phytools' (51).

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Supporting Information Captions

Supplementary Fig. 1. PRISMA reporting diagram for study selection and screening.

Supplementary Table 1. Database breakdown by sample type and detention method. Sample size (records and number of samples), percentage of records with at least one positive sample, and average prevalence by detection method, sample type, and viral subfamily.

Supplementary Table 2. Database breakdown by type of paramyxovirus. Number of studies, host species, and samples tested for different paramyxoviruses, with positivity rates reported for serological and PCR detection attempts as well as number of successful viral isolations.

Supplementary Table 3. Database breakdown by country. Number of studies, number of samples, and mean viral prevalence and seropositivity rates for all countries.

Supplementary Table 4. Results from PACo cophylogenetic analysis of paramyxoviruses in Pteropodidae. Jackknifed squared residuals and upper 95% confidence intervals for each known pteropodid–paramyxovirus link from PACo.