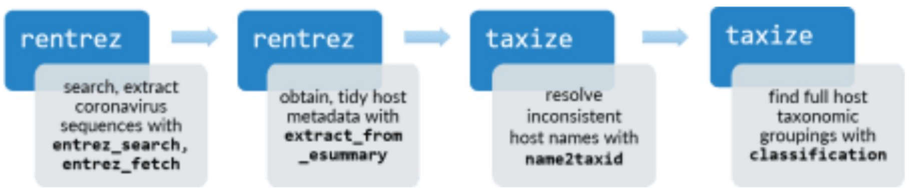
Emerging viruses might be on everyone's mind right now, but as an epidemiologist and disease ecologist I've always been interested in how and why pathogens move from animal hosts to humans.
The current pandemic of the disease we call COVID-19 is caused by *Severe acute respiratory syndrome (SARS) coronavirus 2* (SARS-CoV-2), a virus that has emerged from wildlife like SARS coronavirus and Middle East respiratory syndrome (MERS) coronavirus did previously. Although these viruses are now widely known, there are many more coronaviruses out there in nature, many of which we know little about.

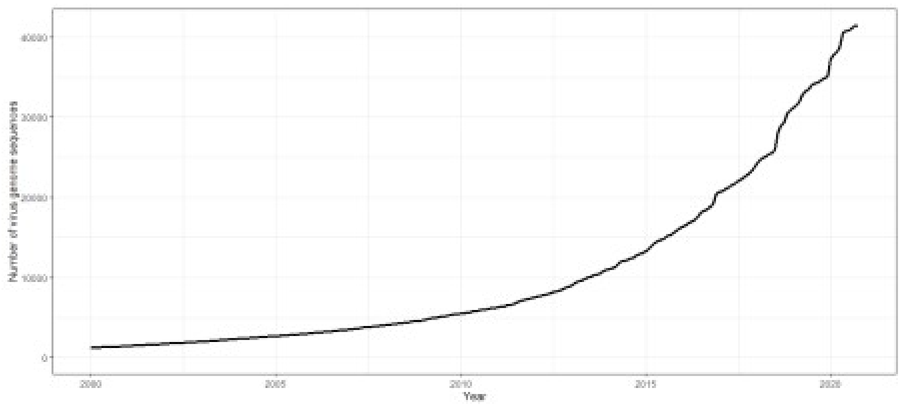
There's perhaps no better time to dive into understanding where viruses like these originate. Genome sequence repositories represent a wealthy source of data to understand the diversity of viruses and their relationships with their hosts.
However, the sheer volume of data and lack of standardisation for some features can make it challenging to use these repositories in practice.
In this post, I'll demonstrate the use of the rentrez and taxizedb packages to search, retrieve and resolve information about genetic sequences of coronaviruses and their animal hosts – and ask which host species have the most coronavirus sequences been sampled from?
Here's a high-level summary of the functions we'll use.


rentrez
search, extract coronavirus sequences with entrez_search, entrez_fetch
rentrez
obtain, tidy host metadata with extract_from _esummary
taxize
resolve inconsistent host names with name2taxid
taxize
find full host taxonomic groupings with classification

Flow diagram of functions used in each package to identify hosts associated with coronavirus sequences

I'll focus on NCBI's GenBank[1], an open-access repository where sequences are submitted and described by individual users.
We can look at the exponential rise in viral genome sequences by importing the release dates of those indexed by NCBI's Genome Reports and plotting a cumulative curve.

```
library(dplyr)
library(ggplot2)
library(magrittr) # load magrittr for use of the pipe operator (%>%)
genbank_seqs <- read.table("ftp://ftp.ncbi.nlm.nih.gov/genomes/GENOME_
REPORTS/viruses.txt",
sep = "\t", header = TRUE, comment.char = "!", quote = "", fill = TRUE)
monthly_seqs <- genbank_seqs$Release.Date %>%
as.Date(format="%Y/%m/%d") %>%
cut("month") %>%
table() %>%
as.data.frame() %>%
rename(month = ".", total = Freq) %>%
mutate(month = as.Date(month), total = cumsum(total))
ggplot(monthly_seqs, aes(month, total)) +
geom_line(lwd=1.4) +
xlab('Year') +
ylab("Number of virus genome sequences") +
scale_x_date(limits = as.Date(c('2000-01-01',Sys.Date()))) +
theme_bw()
```



Viral genome sequence availability between 2000 and present

If we were interested in a small set of viruses, for example if we wanted to construct a specific phylogeny, we might reasonably be able to select and filter these genome sequences manually through web interfaces.
In fact, a powerful new interface of the NCBI Virus front-end to GenBank was unveiled for this purpose earlier this year.
However, if we want to investigate broader viral diversity (or use a data-hungry method like neural networks), we'd benefit from a more reproducible, automated approach.

Obtaining virus sequences with rentrez

Searching sequences

rentrez[2] is a package by David Winter designed to interface with various NCBI databases (collectively known as entrez), including GenBank.
As such, it can not only conduct search requests in a single database, but also cross-reference them (see the vignette for some nice examples of this).
For now let's simply try and find whether there are sequences available for *Pangolin coronavirus* in GenBank's nucleotide sequence database (i.e., `db ="nuccore"`).
This virus recently received a lot of attention because its spike protein (which is thought to be important in determining host range) is very similar to that of SARS-CoV-2[3,4].
We can use `entrez_search()` to conduct a general search across all fields.

```
library(rentrez)
pang_cov_ids <- entrez_search(db="nuccore", term="Pangolin
coronavirus")

Registered S3 method overwritten by 'httr':
method from
print.cache_info hoardr

pang_cov_ids
```

```
Entrez search result with 14 hits (object contains 14 IDs and no
web_history object)
Search term (as translated): "Pangolin coronavirus"[Organism] OR
Pangolin coron ...
```

Looks like there are 14 available genome sequences for this virus -
note that this query doesn't return the actual sequences, but the specific entry IDs.
If we want to directly retrieve sequences, we can use `entrez_fetch()`, referencing these Pangolin coronavirus IDs.
Here I've set `rettype` to output the sequences in FASTA format, a standard bioinformatics format readable by many other packages/software.

```
pang_cov_seqs <- entrez_fetch(db="nuccore", id = pang_cov_ids$ids,
rettype = "fasta_cds_na")
# trim the FASTA string to show first protein coding nucleotide
sequence as an example
gsub("\n\n.*", "", pang_cov_seqs)
```

```
[1] ">lcl|MT799526.1_cds_QLR06869.1_1 [gene=S] [protein=surface
glycoprotein] [protein_id=QLR06869.1] [location=1..3798] [gbkey=CDS]\
nATGTTGTTTTTCTTCTTTTTACACTTTGCCTTAGTAAATTCACAATGTGTTAATTTAAC
AGGTAGAGCTG\nCTATCCAGCCTTCATTCACCAATTCCTCT
CAAAGAGGTGTTTATTATCCTGACACCATATTTAGATCAAA\
nCACACTTGTGTTGAGTCAGGGTTACTTTTTACCTTTTTATTCTAATGTTAGCTGGTATT
ATGCATTGACA\nAAAACTAACAGTGCTGAAAAGAGAGTTGA
TAACCCTGTTTTGGATTTCAAAGACGGTATTTACTTTGCTG\
nCAACTGAAAAATCTAACATTGTCAGAGGTTGGATCTTTGGAACGACTCTTGACAACACA
TCACAGTCACT\nTTTGATAGTTAACAACGCAACTAATGTTA
TCATCAAAGTTTGTAATTTCCAGTTTTGTTATGACCCTTAC\
nCTTAGTGGTTATTATCATAACAATAAAACGTGGAGCACGAGAGAGTTTGCTGTTTATTC
CTCTTATGCCA\nATTGCACTTTTGAGTATGTGTCTAAGTCT
TTTATGCTAGATATAGCTGGCAAAAGTGGCTTATTTGACAC\
nATTAAGAGAGTTTGTTTTCCGAAATGTCGACGGATATTTCAAGATTTACTCAAAATACA
CACCTGTTAAT\nGTAAATAGTAATTTACCTATAGGTTTTTC
AGCACTTGAACCTCTTGTTGAAATTCCAGCTGGCATAAATA\
nTTACTAAATTTAGAACACTCCTCACTATACATAGAGGAGACCCCATGCCTAATAATGGC
TGGACAGTCTT\nTTCAGCTGCTTATTACGTGGGCTATTTAG
CTCCACGTACATTTATGTTAAATTATAATGAAAATGGTACA\
nATAACAGATGCTGTTGATTGTGCCCTAGATCCTCTATCTGAGGCTAAATGCACATTAAA
ATCCTTAACTG\nTTGAAAAAGGAATCTATCAGACTTCTAAC
TTTAGAGTTCAACCAACTGAATCTATAGTTAGGTTTCCAAA\
nTATTACAAACTTATGCCCTTTTGGTGAAGTTTTCAATGCAACCACTTTTGCATCTGTTT
ATGCTTGGAAT\nAGAAAGAGAATCAGTAACTGTGTTGCTGA
TTACTCTGTTCTTTACAACTCCACTTCTTTCTCAACATTCA\
nAATGTTATGGAGTTTCACCAACCAAACTAAATGATCTCTGCTTTACTAACGTTTATGCA
GACTCATTTGT\nAGTTAGAGGTGATGAAGTCAGACAAATTG
CTCCAGGACAAACAGGAAGAATTGCTGACTATAATTATAAA\
nCTCCCTGATGATTTCACAGGTTGTGTAATAGCTTGGAATTCTAACAACCTTGATTCTAA
GGTTGGTGGTA\nATTATAACTACCTTTATAGATTGTTTAGA
AAGTCCAACCTCAAACCTTTTGAACGAGACATTTCTACAGA\
nAATATACCAAGCTGGTAGTACACCCTGCAATGGGGTTGAAGGTTTTAACTGTTACTTTC
```

```
CTCTACAATCT\nTATGGTTTCCACCCTACTAATGGTGTTGG
TTACCAACCTTATAGAGTAGTAGTATTGTCATTTGAACTTT\
nTAAATGCACCTGCTACTGTTTGTGGACCTAAACAGTCCACTAACCTAGTTAAAAACAAA
TGTGTCAACTT\nCAATTTTAATGGTCTAACAGGCACAGGTG
TTCTTACAGAGTCTAGCAAAAAGTTTTTGCCTTTCCAACAA\
nTTTGGCAGAGATATTGCCGACACTACTGATGCTGTCCGTGATCCACAGACACTTGAAAT
TCTTGATATCA\nCACCGTGTTCTTTTGGTGGTGTCAGTGTT
ATAACACCAGGAACAAACACTTCTAACCAAGTGGCTGTTCT\
nTTATCAGGATGTTAACTGCACTGAAGTCCCTGTTGCTATTCATGCAGATCAATTAACAC
CAACCTGGCGT\nGTTTACTCTACAGGTTCAAATGTTTTTCA
AACGCGTGCAGGCTGTTTAATAGGGGCTGAACATGTTAACA\
nACACTTACGAGTGTGACATACCAATTGGTGCAGGAATATGTGCCAGTTATCAGACTCAA
ACTAATTCACG\nTAGTGTTTCAAGTCAAGCTATTATTGCCT
ACACTATGTCACTTGGTGCAGAAAATTCAGTTGCTTATGCT\
nAATAACTCTATTGCCATACCTACAAATTTTACTATTAGTGTGACCACTGAAATTCTACC
AGTGTCTATGA\nCAAAGACATCAGTAGATTGTACAATGTAC
ATTTGTGGTGACTCAATAGAGTGCAGCAACCTTTTGCTCCA\
nATATGGTAGTTTTTGCACACAACTTAATCGTGCTTTAACTGGAATTGCTGTTGAACAAG
ACAAAAACACA\nCAGGAAGTTTTTGCACAAGTTAAACAAAT
TTACAAGACACCACCAATAAAGGATTTTGGTGGTTTCAACT\
nTTTCTCAAATATTACCAGATCCATCAAAACCAAGCAAGAGGTCATTTATTGAAGATTTA
CTCTTCAACAA\nAGTGACACTTGCTGATGCTGGCTTCATCA
AACAATATGGTGATTGCCTTGGTGATATTGCCGCTAGAGAT\
nCTTATTTGTGCACAAAAGTTTAATGGCCTTACTGTTCTGCCACCTTTGCTCACAGATGA
AATGATTGCTC\nAATACACCTCTGCACTACTTGCAGGGACA
ATCACATCAGGTTGGACCTTTGGTGCTGGTGCAGCATTACA\
nGATACCATTTGCTATGCAAATGGCTTATAGGTTTAATGGTATTGGAGTTACACAAAATG
TTCTCTACGAG\nAACCAAAAACTAATTGCAAACCAATTCAA
CAGTGCAATTGGCAAAATTCAAGATTCACTTTCATCTACTG\
nCAAGTGCACTTGGAAAACTTCAAGATGTTGTCAACCAAAATGCACAGGCTTTAAACACA
CTTGTTAAACA\nACTCAGCTCTAATTTTGGAGCCATTTCGA
GTGTGTTAAATGACATTCTTTCACGTCTTGACAAAGTTGAG\
nGCTGAAGTCCAAATTGACAGGTTGATCACTGGCAGATTACAAAGTTTGCAGACATACGT
GACTCAACAAC\nTAATTAGAGCCGCAGAAATTAGAGCTTCT
GCTAATCTTGCCGCAACTAAGATGTCTGAATGTGTTCTTGG\
nACAATCTAAAAGAGTTGACTTTTGTGGTAAAGGCTACCACCTTATGTCTTTTCCGCAGT
CAGCACCTCAT\nGGTGTAGTCTTTTTGCATGTGACTTATGT
TCCATCTCAAGAAAAGAATTTTACTACTACCCCTGCCATTT\
nGTCATGAAGGAAAAGCACACTTTCCTCGTGAAGGTGTTTTCGTTTCAAACGGCACGCAC
TGGTTTGTAAC\nACAAAGGAATTTCTATGAACCACAAATTA
TTACCACGGACAATACTTTTGTCTCTGGTAGCTGTGATGTT\
nGTGATTGGAATTGTCAACAACACAGTTTATGATCCTTTGCAACCAGAACTTGATTCATT
CAAGGAGGAGT\nTGGACAAATATTTTAAAAATCATACATCA
CCAGATGTTGATTTAGGTGACATTTCTGGCATCAACGCTTC\
nAGTTGTCAACATTCAGAAAGAAATTGACCGCCTCAACGAGGTTGCCAAAAATCTAAATG
AATCTCTCATC\nGACCTCCAAGAACTTGGAAAGTATGAGCA
GTATATAAAATGGCCATGGTATATTTGGCTAGGATTTATTG\
nCAGGCTTGATAGCTATAATCATGGTTACAATCATGTTATGCTGTATGACCAGTTGCTGC
AGTTGTCTCAA\nGGGCTGTTGTTCTTGTGGCTCCTGCTGTA
AATTTGATGAAGACGACTCTGAGCCAGTACTCAAAGGAGTC\nAAATTACATTACACATAA"
```

Understanding sequence-associated metadata

However, we want to access more information about these viruses than just their nucleotide sequence.
We might want to find out information from the entry's title, or filter out incomplete sequences, etc.
`entrez_summary()` can retrieve metadata and features of the sequence as an object of class `esummary_list`.
Then we can use `extract_from_esummary()` to pull out the metadata of interest as a vector (or data frame).
Let's find out when these Pangolin coronavirus sequences were uploaded to GenBank.

```
pang_cov_summary <- entrez_summary(db="nuccore", id = pang_cov_ids$ids)
pang_cov_summary

List of 14 esummary records. First record:
$`1879599858`
esummary result with 31 items:
[1] uid caption title extra gi createdate updatedate flags taxid slen
[11] biomol moltype topology sourcedb segsetsize projectid genome
subtype subname assemblygi
[21] assemblyacc tech completeness geneticcode strand organism strain
biosample statistics properties
[31] oslt

extract_from_esummary(pang_cov_summary, elements = c("createdate")) %>%
table()


.
2020/02/20 2020/04/01 2020/05/18 2020/08/01
1 6 1 6
```

Looks like these sequences were mostly uploaded in April and August!
Note that this upload date field does not represent the *sampling* date (this can be found within the sub-features; see below)

More CoVs, more problems

That example's nice and simple, but in practice, if we're accessing these data programmatically we've probably got a much bigger scale of dataset in mind.
Since they're so diverse, we could look at how many sequences there are across all coronaviruses.
This time, I'll use the ID corresponding to the family *Coronaviridae* in NCBI's Taxonomy (`txid11118`) and search for this in the **'Organism'** field (`[Organism]`).
By default, this will return matches for this taxonomic ID, plus any taxonomic IDs below it - meaning we're searching for all coronavirus species (should you not wish to return matches of nested taxa, use `[Organism:noexp]` instead).

```
all_cov_ids <- entrez_search(db="nuccore", term="txid11118[Organism]",
retmax = 70000)
all_cov_ids

Entrez search result with 63225 hits (object contains 63225 IDs and no
web_history object)
Search term (as translated): txid11118[Organism]
```

That's a lot of coronavirus sequences! By default, `entrez_search()` returns only 20 IDs, so I increased `retmax` to make sure all of them are captured.
This is also a good forewarning that there are reasonable limits to usage of eUtils (the API we're accessing underneath) and for very large queries with `entrez_fetch()` or `entrez_summary()`, we should break them up into smaller individual queries.
I've done this by looping `entrez_summary()` across our IDs and joining the results together as a single function.

```
get_metadata <- function(x){
query_index <- split(seq(1,length(x)), ceiling(seq_along(seq(1,
length(x)))/300))
Seq_result <- vector("list", length(query_index))
for (i in 1:length(query_index)) {
Seq_result[[i]] <- entrez_summary(db = "nuccore",id =
x[unlist(query_index[i])])
Sys.sleep(1)
}
if(length(x) == 1){
return(Seq_result)
} else {
return(Seq_result %>%
purrr::flatten() %>%
unname() %>%
structure(class = c("list", "esummary_list"))) # coerce to esummary
list
}
}
all_cov_summary <- get_metadata(all_cov_ids$ids)
```

By using `Sys.sleep()`, this function also delays the speed at which queries are sent to comply with the eUtils API usage limits.
If you're likely to conduct lots of queries, you can set up an API key to increase your usage and use the web history feature to quickly revisit previous queries.

Now we have metadata for all coronavirus sequences, let's investigate what host animals these coronaviruses came from.
You may have noticed there wasn't an explicit "host" feature among the metadata we got in our previous esummary result for Pangolin coronavirus.
This is because host is considered a sub-feature of GenBank records and (if present) it's contained in a bar-delimited string under `subtype`.
Processing this data to a tidy format requires a couple of extra steps - I've simply split these strings and reconstructed them as a data frame, adding the accession version as a unique sequence identifier.

```
all_accession <- extract_from_esummary(all_cov_summary, elements =
c("accessionversion"))
all_subname_list <- extract_from_esummary(all_cov_summary, elements =
c("subname", "subtype"),
simplify=FALSE)
all_subname_list[[3000]] # show an example of sub-features

$subname
[1] "bat-SL-CoVZXC27|Rhinolophus pusillus|China|Feb-2017"
$subtype
[1] "isolate|host|country|collection_date"

all_subname_list <- all_subname_list %>%
lapply(function(x) {
subname_row <- x["subname"] %>%
stringr::str_split("\\|") %>%
unlist() %>%
matrix(nrow = 1, byrow = FALSE) %>%
as.data.frame()
subtype_names <- x["subtype"] %>%
stringr::str_split("\\|") %>%
unlist()
set_colnames(subname_row, subtype_names)
})
all_metadata_df <- data.frame(accession = all_accession,
suppressMessages(bind_rows(all_subname_list)))
str(all_metadata_df[,1:21]) # show only the first 20 sub-features;
there are many fields!

'data.frame': 63225 obs. of 21 variables:
$ accession : chr "MW075808.1" "MW075807.1" "MW075806.1" "MW075805.1"
...
$ isolate : chr "SARS-CoV-2/human/USA/NMDOH-2020315962/2020" "SARS-
CoV-2/human/USA/NMDOH-2020315509/2020" "SARS-CoV-2/human/USA/NMDOH-
2020315508/2020" "SARS-CoV-2/human/USA/NMDOH-2020315317/2020" ...
$ host : chr "Homo sapiens" "Homo sapiens" "Homo sapiens" "Homo
sapiens" ...
$ gb_acronym : chr "SARS-CoV-2" "SARS-CoV-2" "SARS-CoV-2" "SARS-CoV-2"
...
$ country : chr "USA: New Mexico" "USA: New Mexico" "USA: New Mexico"
"USA: New Mexico" ...
$ isolation_source : chr "nasopharyngeal swab" "nasopharyngeal swab"
"nasopharyngeal swab" "nasopharyngeal swab" ...
$ collection_date : chr "2020-08-27" "2020-08-27" "2020-08-27"
"2020-08-27" ...
$ collected_by : chr NA NA NA NA ...
$ strain : chr NA NA NA NA ...
$ note : chr NA NA NA NA ...
$ chromosome : chr NA NA NA NA ...
$ old_name : chr NA NA NA NA ...
$ genotype : chr NA NA NA NA ...
$ serotype : chr NA NA NA NA ...
$ clone : chr NA NA NA NA ...
$ lab_host : chr NA NA NA NA ...
$ identified_by : chr NA NA NA NA ...
$ lat_lon : chr NA NA NA NA ...
$ ...1 : chr NA NA NA NA ...
$ acronym : chr NA NA NA NA ...
$ metagenome_source: chr NA NA NA NA ...
```

Immediately we see that not every sub-feature is filled.
In fact most are rarely ever used.
Let's take a look at the 15 most common hosts of coronaviruses according to the host sub-features.

```
host_table <- all_metadata_df %>%
filter(!is.na(host)) %>%
count(host) %>%
arrange(-n)
host_table %>%
slice(1:15)

 host n
1 Homo sapiens 28049
2 chicken 5180
3 pig 2042
4 Gallus gallus 1374
5 porcine 1359
6 swine 1339
7 Felis catus 950
8 Sus scrofa 494
9 Camelus dromedarius 477
10 cat 440
11 Microchiroptera 433
12 Eidolon helvum 394
13 piglet 290
14 Canis lupus familiaris 251
15 duck 244
```

As you might expect, most nucleotide sequences are sampled from humans or domestic animals, particularly chickens and pigs, as there are several non-human coronaviruses that cause significant outbreaks in these animals each year.
In fact, the only wild animals in this top 15 are bats: *Eidolon helvum*, an African fruit bat, and the rather unspecific "Microchiroptera", which we'll decipher shortly.

However, because GenBank entries are filled out by the individuals depositing the data, their metadata can use inconsistent terms.
Among these top 15 hosts, we find *pig*, *swine*, *Sus scrofa*, and *piglet*, all of which describe the same animal.
The same is true for *chicken* and *Gallus gallus*!

Standardising taxonomies with taxizedb

This is a problem the package taxizedb[5] can help solve.
This is a version of the taxize package by Scott Chamberlain and Zebulun Arendsee.
Its functionality is essentially similar to taxize.
The key difference is that rather than making web queries, it references a locally stored taxonomy database, so you're not limited by API restrictions or web connectivity.

The first time you use taxizedb, you'll need to use the corresponding function to download the taxonomy database(s) you'll use.
We'll stick with NCBI's taxonomy to keep IDs consistent.
The function `db_download_ncbi()` will set everything up, though be warned this generates a fairly hefty file at approx. 2GB!

Once we have a local taxonomy database, we're ready to start resolving using `name2taxid()`, which converts various common names and synonyms to their lowest specific taxonomic ID (just like the ID we used for *Coronaviridae*, and if we wanted, we could equally use these taxonomic IDs to search for nucleotide sequences of hosts!)

```
library(taxizedb)
src_ncbi <- src_ncbi() # load the NCBI taxonomy database into R
all_tax_match <- host_table$host %>%
name2taxid(db = "ncbi", out_type = "summary")
all_tax_match

# A tibble: 422 x 2
name id

1 Homo sapiens 9606
2 chicken 9031
3 pig 9823
4 Gallus gallus 9031
5 swine 9823
6 Felis catus 9685
7 Sus scrofa 9823
8 Camelus dromedarius 9838
9 cat 9685
10 Microchiroptera 30560
# ... with 412 more rows
```

Great - our synonymous strings for chickens and pigs have been matched to the same ID!
Let's also use these taxonomic ID matches to check out what is meant by the unspecific term "Microchiroptera".
Using NCBI's taxonomy web interface, ID 30560 seems to correspond to a sub-order grouping within the bat order *Chiroptera*.
However, we can just as easily examine this through taxizedb.
`classification()` will return the taxonomic classification **above** the given ID, while `downstream()` will return the taxonomic classifications **below** the given ID, branching down to a specific level - so we can see some example species considered "Microchiroptera"!

```
classification(30560, db = "ncbi")

$`30560`
name rank id
1 cellular organisms no rank 131567
```

```
2 Eukaryota superkingdom 2759
3 Opisthokonta no rank 33154
4 Metazoa kingdom 33208
5 Eumetazoa no rank 6072
6 Bilateria no rank 33213
7 Deuterostomia no rank 33511
8 Chordata phylum 7711
9 Craniata subphylum 89593
10 Vertebrata no rank 7742
11 Gnathostomata no rank 7776
12 Teleostomi no rank 117570
13 Euteleostomi no rank 117571
14 Sarcopterygii superclass 8287
15 Dipnotetrapodomorpha no rank 1338369
16 Tetrapoda no rank 32523
17 Amniota no rank 32524
18 Mammalia class 40674
19 Theria no rank 32525
20 Eutheria no rank 9347
21 Boreoeutheria no rank 1437010
22 Laurasiatheria superorder 314145
23 Chiroptera order 9397
24 Microchiroptera suborder 30560
attr(,"class")
[1] "classification"
attr(,"db")
[1] "ncbi"

downstream(30560, db = "ncbi", downto = "species")

$`30560`
childtaxa_id childtaxa_name rank
1 2715946 Miniopterus paululus species
2 2695401 Murina rongjiangensis species
3 2695400 Murina jinchui species
4 2695399 Murina harrisoni species
5 2664497 Doryrhina camerunensis species
6 2664495 Scotoecus hindei species
7 2664494 Chaerephon bivittatus species
8 2664386 Eptesicus velatus species
9 2664385 Eptesicus montanus species
10 2664384 Eptesicus magellanicus species
11 2664383 Eptesicus macrotus species
12 2614833 Pipistrellus cf. coromandra RM-2019 species
13 2608643 Pipistrellus cf. grandidieri JJA-2019 species
14 2608642 Parahypsugo happoldorum species
15 2608641 Neoromicia isabella species
16 2608640 Parahypsugo bellieri species
17 2603869 Macronycteris vittatus species
18 2603867 Rhinolophus willardi species
19 2603866 Rhinolophus rhodesiae species
20 2603865 Rhinolophus lobatus species
```

```
21 2603864 Rhinolophus kahuzi species
22 2603863 Rhinolophus gorongosae species
23 2603862 Rhinolophus deckenii species
24 2603861 Rhinolophus cf. landeri TD-2019 species
25 2603860 Rhinolophus cf. denti/simulator TD-2019 species
26 2603859 Rhinolophus cf. denti TD-2019 species
[ reached 'max' / getOption("max.print") -- omitted 1021 rows ]
attr(,"class")
[1] "downstream"
attr(,"db")
[1] "ncbi"
```

Of course, as host metadata is often individually typed, this approach isn't infallible – typos, plus any strings that are very colloquial or ambiguous, e.g., "broiler", or overly detailed, e.g., "Felis catus (cat); breed Scottish Fold; color red" may need some manual resolution.

Where have coronaviruses been found?

Since we now have a reproducible way of retrieving the full taxonomic classifications of each host, we can now finally tie all this together and ask which hosts have the most coronavirus sequences on GenBank.
Firstly, we'll look up the `classification()` of all hosts and restructure the output as a name column and an ID column per taxonomic level from class to species.

```
all_tax_full <- all_tax_match %>%
bind_cols(
all_tax_match$id %>%
classification(db = "ncbi") %>%
lapply(function(x) {
x %>%
data.frame() %>%
distinct() %>%
filter(rank == "class" | rank == "order" | rank == "family" |
rank == "genus" | rank == "species") %>%
reshape2::melt(id = "rank") %>%
tidyr::unite(col = rank_var, c("rank", "variable")) %>%
tidyr::spread(key = rank_var, value = value)
}) %>%
bind_rows() %>%
select(class_name, class_id, order_name, order_id, family_name,
family_id,
genus_name, genus_id, species_name, species_id)
)
all_tax_full

# A tibble: 422 x 12
name id class_name class_id order_name order_id family_name family_id
genus_name genus_id species_name species_id

1 Homo sapiens 9606 Mammalia 40674 Primates 9443 Hominidae 9604 Homo
```

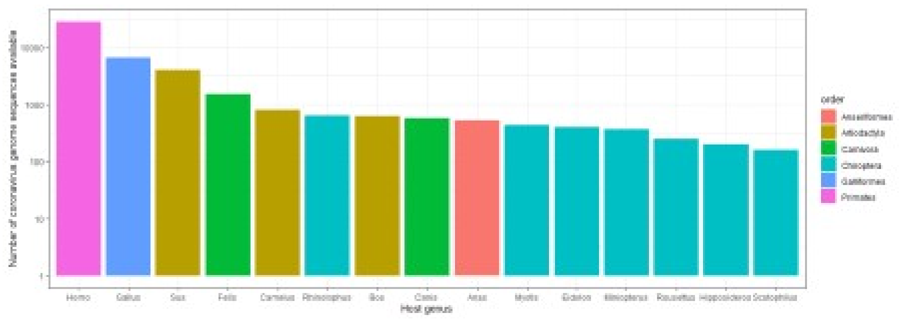
```
9605 Homo sapiens 9606
2 chicken 9031 Aves 8782 Galliformes 8976 Phasianidae 9005 Gallus 9030
Gallus gallus 9031
3 pig 9823 Mammalia 40674 Artiodacty~ 91561 Suidae 9821 Sus 9822 Sus
scrofa 9823
4 Gallus gallus 9031 Aves 8782 Galliformes 8976 Phasianidae 9005 Gallus
9030 Gallus gallus 9031
5 swine 9823 Mammalia 40674 Artiodacty~ 91561 Suidae 9821 Sus 9822 Sus
scrofa 9823
6 Felis catus 9685 Mammalia 40674 Carnivora 33554 Felidae 9681 Felis
9682 Felis catus 9685
7 Sus scrofa 9823 Mammalia 40674 Artiodacty~ 91561 Suidae 9821 Sus 9822
Sus scrofa 9823
8 Camelus dromedari~ 9838 Mammalia 40674 Artiodacty~ 91561 Camelidae
9835 Camelus 9836 Camelus dromedari~ 9838
9 cat 9685 Mammalia 40674 Carnivora 33554 Felidae 9681 Felis 9682 Felis
catus 9685
10 Microchiroptera 30560 Mammalia 40674 Chiroptera 9397
# ... with 412 more rows
```

Next, we'll attach these host taxonomic classifications to our sequences by merging these columns in to our metadata.
From here we can count up, say, the 15 most frequent host genera among coronavirus sequences, filtering out any sequences missing host genus information.

```
all_metadata_plot_df <- all_metadata_df %>%
# match each unique sequence to its taxonomic classification
left_join(all_tax_full, by = c("host" = "name")) %>%
filter(!is.na(genus_name)) %>%
count(order_name, genus_name) %>%
arrange(-n) %>%
slice(1:15) %>%
mutate(genus_name = factor(genus_name, levels = genus_name))
```

Lastly, we'll visualise our top 15 genera in a simple barplot using ggplot2, colour-coding each according to which taxonomic order they belong to.

```
 ggplot(all_metadata_plot_df, aes(x = genus_name, y = n, fill =
order_name)) +
geom_bar(stat = "identity") +
scale_y_log10(breaks = c(1, 10, 100, 1000, 10000)) +
xlab('Host genus') +
ylab("Number of coronavirus genome sequences available") +
guides(fill = guide_legend(title="order")) +
theme_bw()
```



Top fifteen most common animal genera represented in coronavirus genome sequence host metadata, colour-coded by order

While there are a tremendous number of sequences of coronaviruses sampled from humans and domestic animals, there are also quite a few genera of bats present among the most represented hosts! Again, this isn't particularly surprising, as bats have a rich coevolutionary history with coronaviruses[6], which has been an intense focus of study.

Conclusion

rentrez offers an automated way to search and retrieve genome sequences and metadata, while taxizedb is capable of retrieving and resolving various levels of taxonomic classification.
Using these packages in combination represents a reproducible approach to extracting a standardised dataset of coronavirus nucleotide sequences and their sample hosts.
This reproducibility is essential for those studies attempting to modelling host-virus relationships to better understand future epidemic threats in humans.
I've used the above functions to process data to a suitable standard for training machine learning models to predict coronavirus hosts based on their sequence features[7].
But of course, neither package is limited to viruses; their functionality makes for some brilliant additions to any biologist's R toolkit!