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Clara Flintrop
Martin Jones



THE UNIVERSITY
of EDINBURGH



Edinburgh Genomics
(especially Marian Thomson)

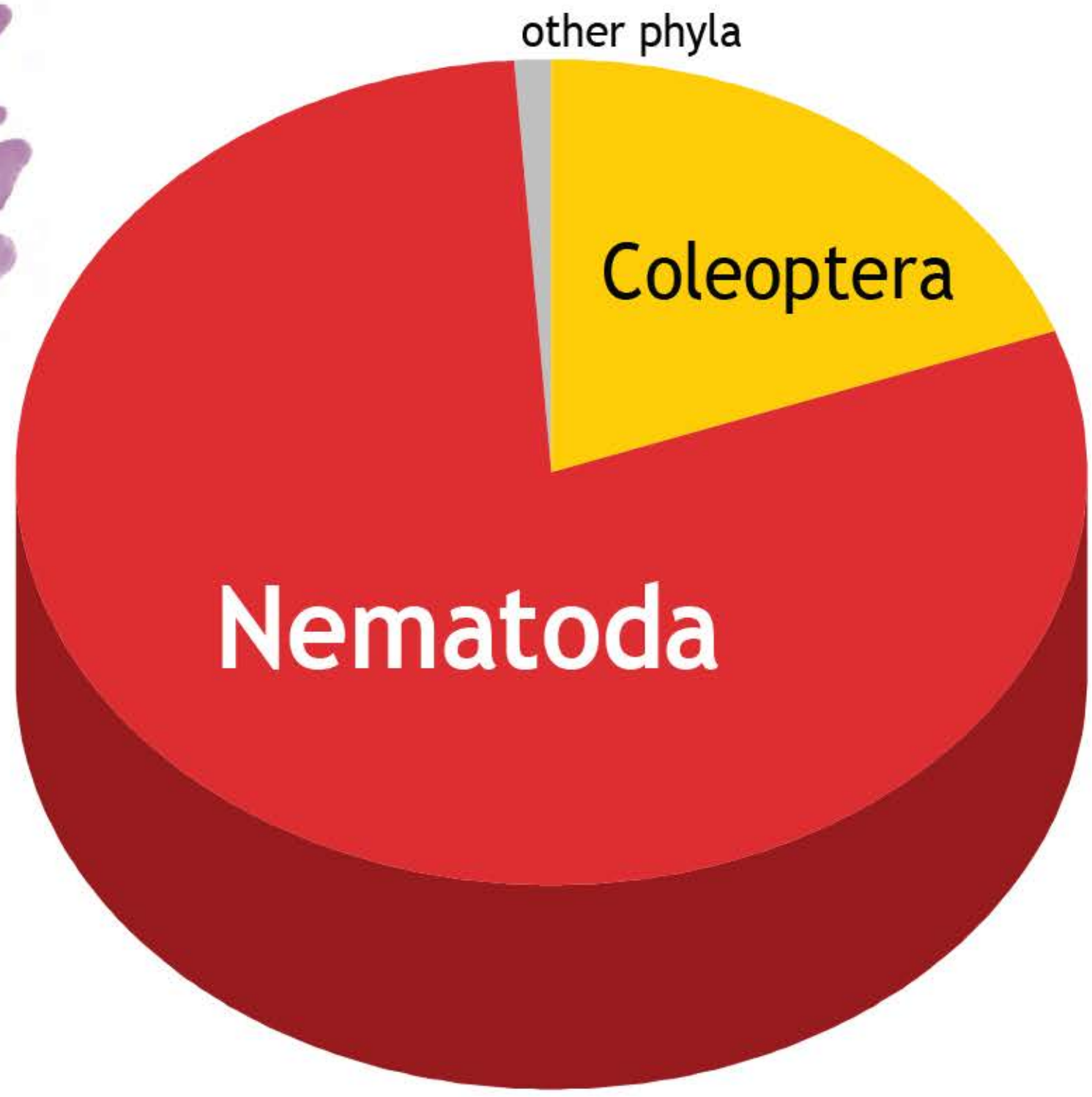
Metabarcoding collaborators

Simon Creer, Vera Fonseca, Kelley Thomas,



"In short, if all the matter in the universe except the nematodes were swept away, our world would still be dimly recognizable, and if, as disembodied spirits, we could then investigate it, we should find its mountains, hills, vales, rivers, lakes, and oceans represented by a film of nematodes. The location of towns would be decipherable, since for every massing of human beings there would be a corresponding massing of certain nematodes. Trees would still stand in ghostly rows representing our streets and highways. The location of the various plants and animals would still be decipherable, and, had we sufficient knowledge, in many cases even their species could be determined by an examination of their erstwhile nematode parasites."

Nathan Cobb 1915





Molecular barcodes for soil nematode identification

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Abstract

Using a molecular barcode, derived from single-specimen polymerase chain reaction (PCR) and sequencing of the 5' segment of the small subunit ribosomal RNA (SSU) gene, we have developed a molecular operational taxonomic unit (MOTU) scheme for soil nematodes. Individual specimens were considered to belong to the same MOTU when the sequenced segment of 450 bases was > 99.5% identical. A Scottish upland *Agrostis-Festuca* grassland soil was sampled, using both culture-based and random selection methods. One hundred and sixty-six cultured isolates were sequenced, and clustered into five MOTU. From 74 randomly sampled individuals across the study site, 19 MOTU were defined. A subsequent sample of 18 individuals from a single subplot contained eight MOTU, four of which were unique to the single subplot sample. Interestingly, seven of these MOTU were not present in the culture-independent sampling. Overall, a total of 23 MOTU were defined from only 240 sequences. Many MOTU could readily be assigned to classical, morphologically defined taxonomic units using a database of SSU sequences from named nematode species. The MOTU technique allows a rapid assessment of nematode taxon diversity in soils. Correlation with a database of sequences from known species offers a route to application of the technique in ecological surveys addressing biological as well as genetic diversity.

Keywords: biodiversity assessment, DNA sequence, nematodes, 18S ribosomal RNA (SSU)

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Introduction

Measurement of meiofaunal diversity and abundance is an important but time consuming process. Morphological identification of individual organisms to named species is often not technically possible due to sheer abundance, small size, and lack of expert knowledge of the groups encountered. This is especially true of nematodes, whose diversity in soils and sediments remains essentially unknown. Surveys of benthic sediments suggest that the total species number for marine nematodes may exceed 1 million (Lambshhead 1993; Lambshhead 2001), with only a few thousand described in the scientific literature (Malakhov 1994; De Ley & Blaxter 2001). In terrestrial systems, nematode diversity appears to be under-reported (Lawton *et al.* 1998), with, for example, only about 200 species of soil nematodes being described from the British Isles (Boag & Yeates 1998). The maximum number of nematode taxa described from a single soil site is 228 from a prairie in Kansas, USA (Orr & Dickerson 1966; Boag &

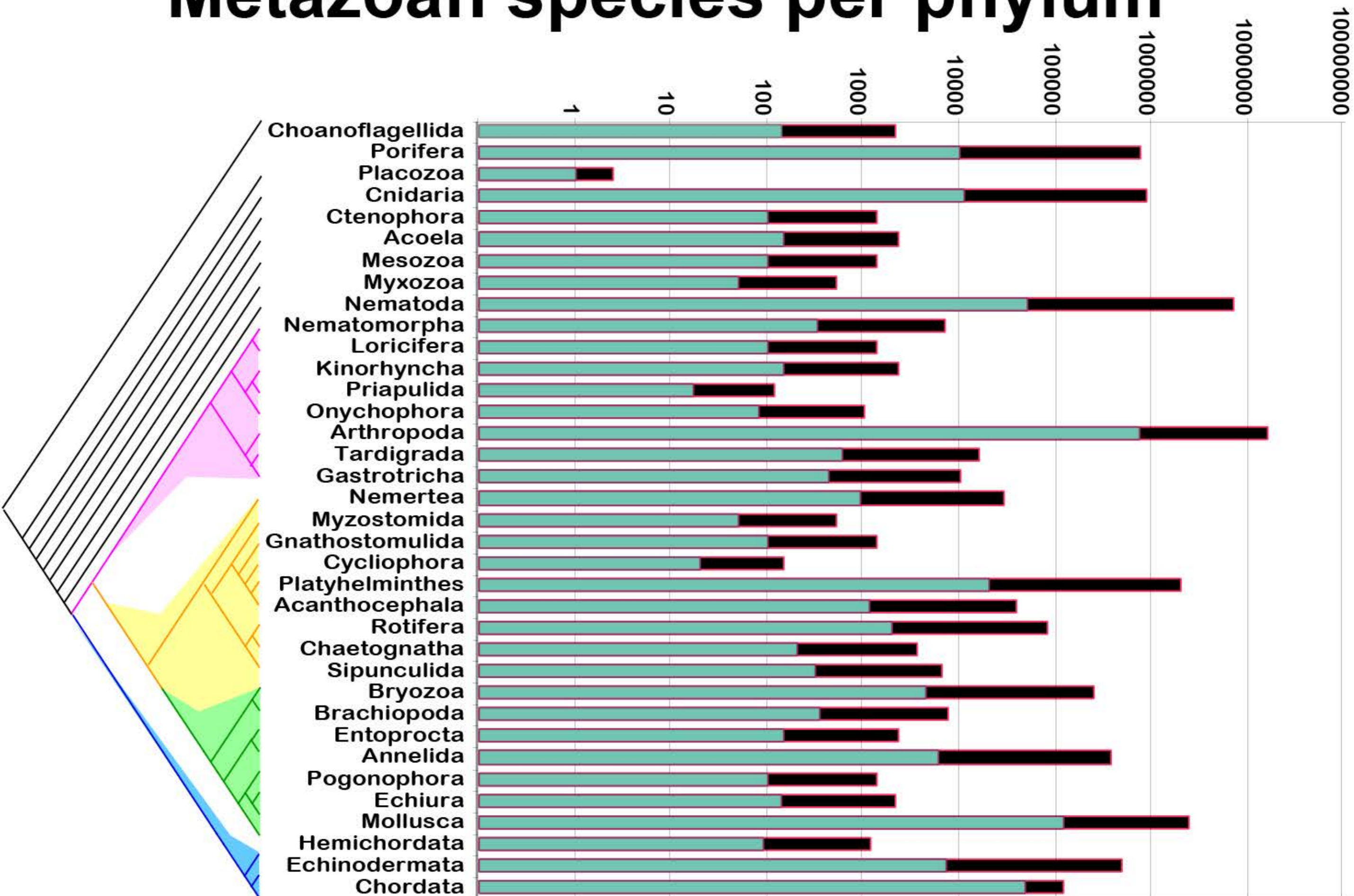
Yeates 1998). Given that many (or most) nematode species have yet to be formally described morphologically (Platt 1994), a robust and transferable system of identification, applicable to all individuals and taxa, is sorely needed.

As terrestrial nematodes can easily exceed one million individuals per square metre of soil, it is likely that any attempt to exhaustively describe a local nematode fauna will become an undertaking of monographic proportions. In addition, many taxa can be diagnosed only from adult male- or female-specific structures, or from population measures of relative morphological characters. In such cases, larvae, individuals of the 'wrong' sex, or individual specimens may not be identifiable. For many studies, identifications are only made to generic level, and taxa are designated as 'genus_x 1', 'genus_x 2'. This precludes simple correlation of surveys carried out by different experts at different sites and times.

We approach this problem from a use-value perspective. We would like to develop a method that is simple, universal and cross-compatible between surveys. We aim to define operational taxonomic units (OTU) relevant to the study at hand. These OTU need not have any formal correlation with published species descriptions, though such

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Metazoan species per phylum



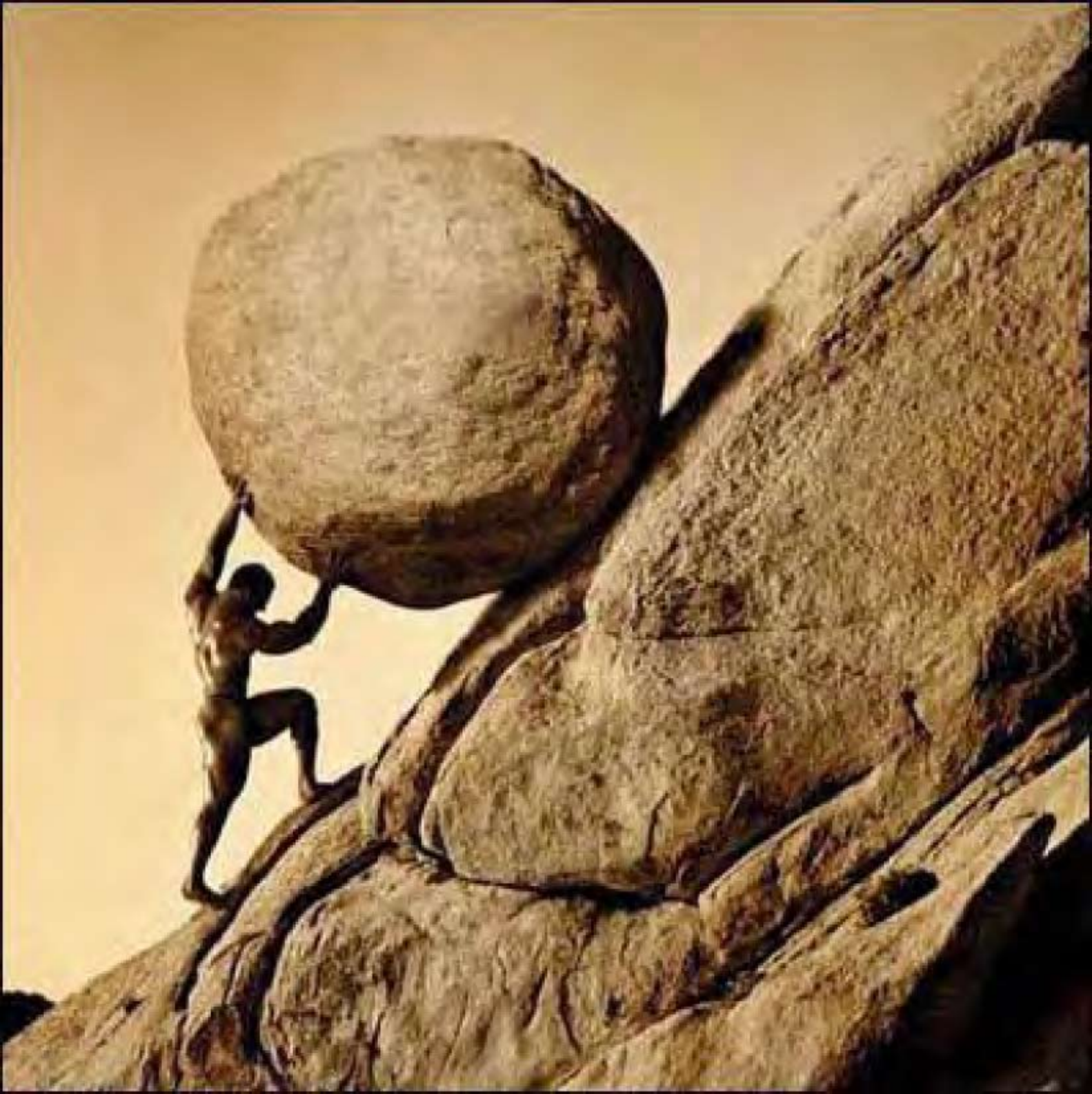
The Walrus and the Carpenter
Were walking close at hand;
They wept like anything to see
Such quantities of sand:
"If this were only cleared away,"
They said, "it would be grand!"

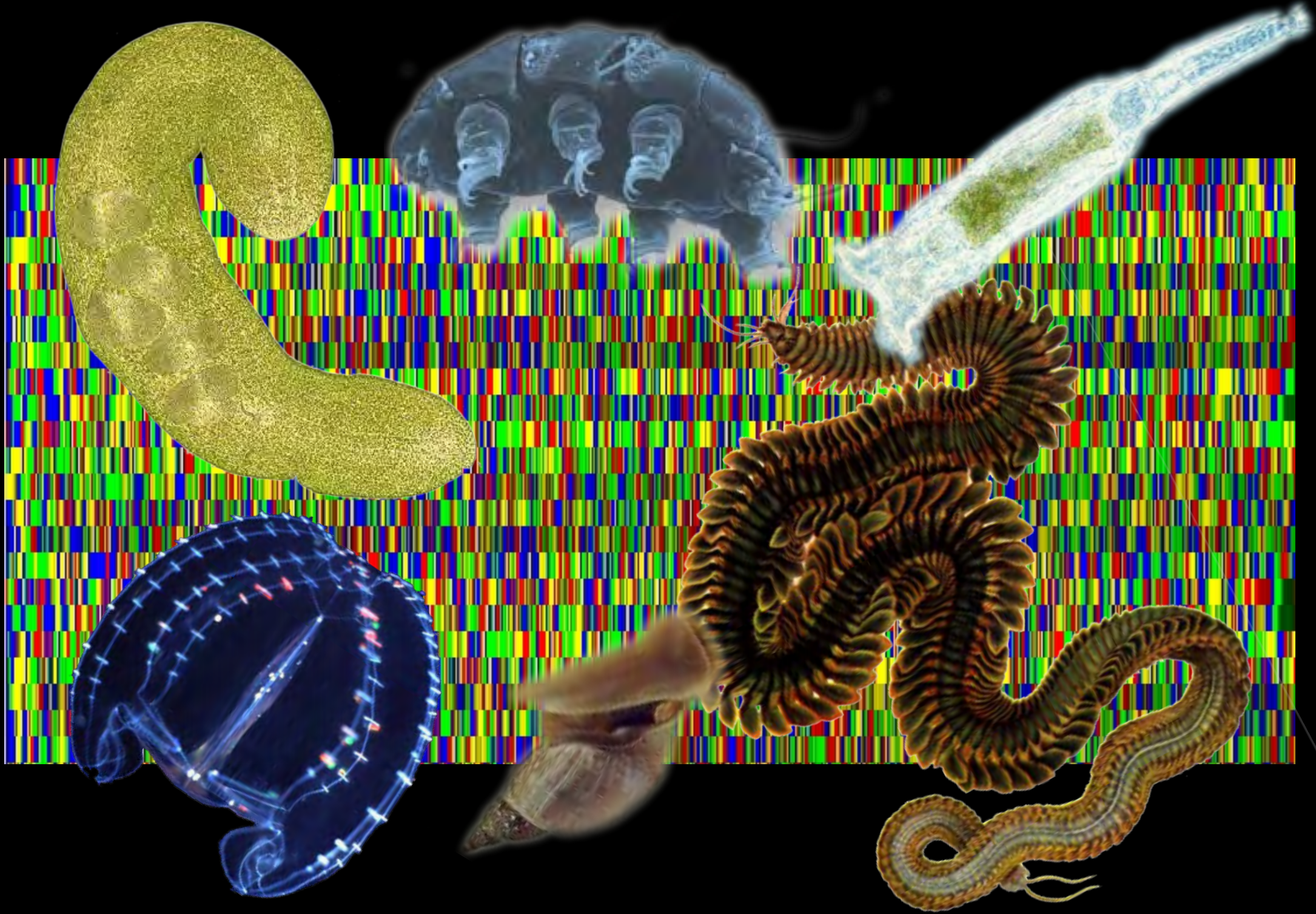


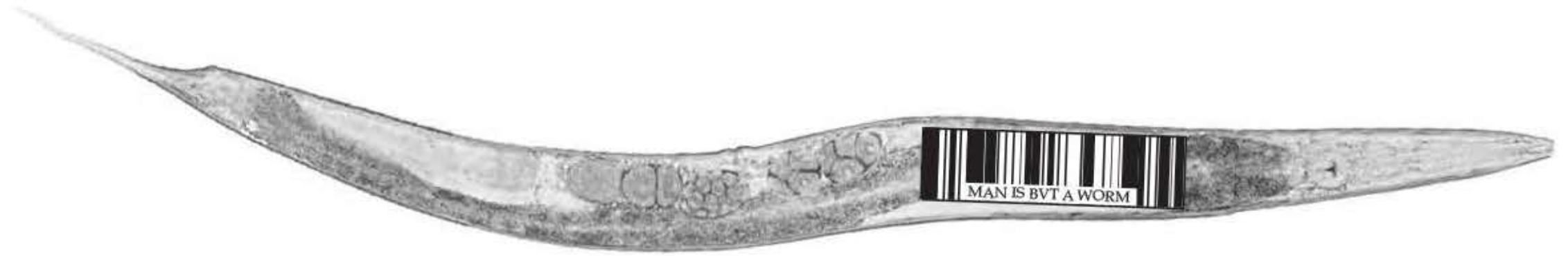
"If seven maids with seven mops
Swept it for half a year.
Do you suppose," the Walrus said,
"That they could get it clear?"
"I doubt it," said the Carpenter,
And shed a bitter tear.

The Walrus and The Carpenter
Lewis Carroll

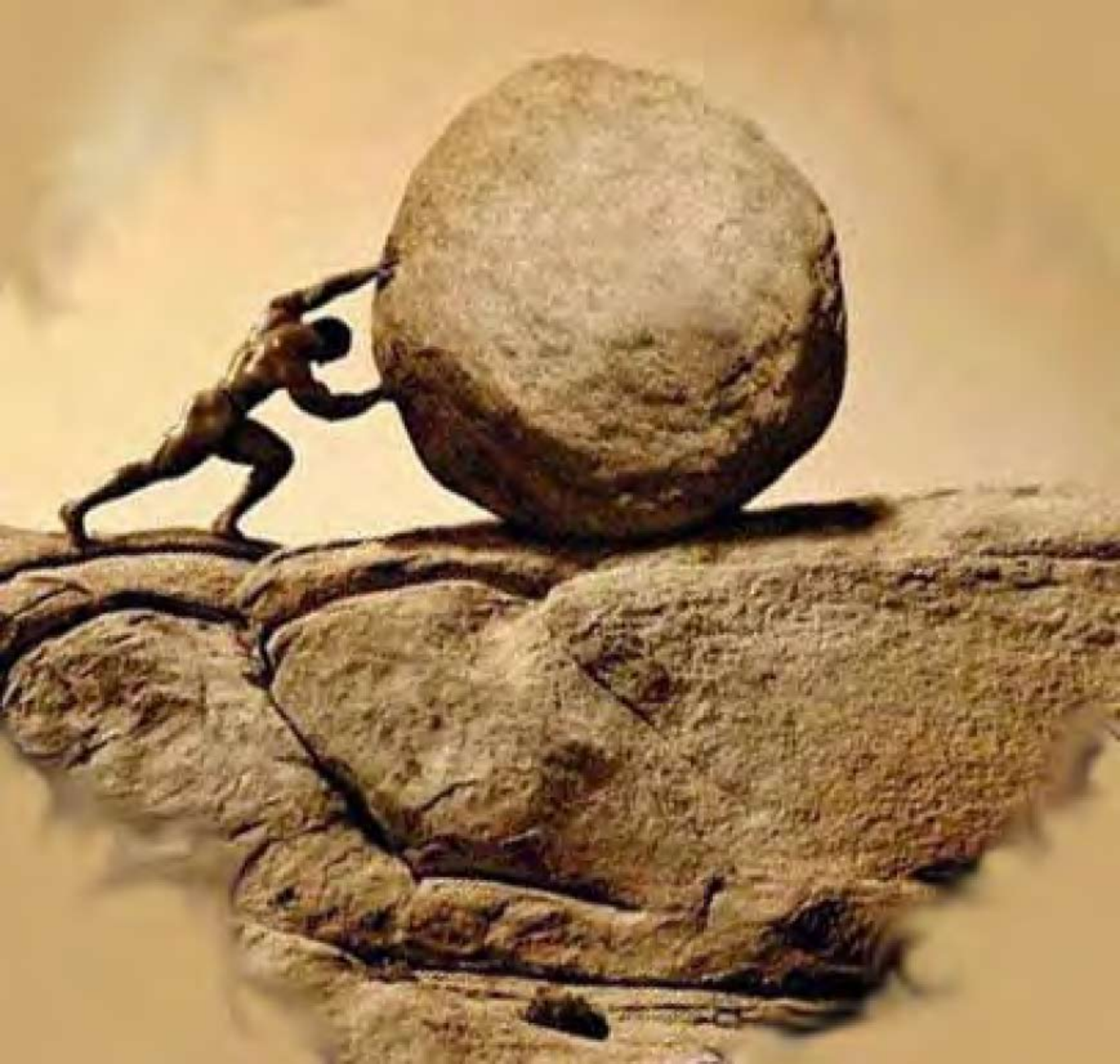
(from *Through the Looking-Glass and What Alice Found There*, 1872)

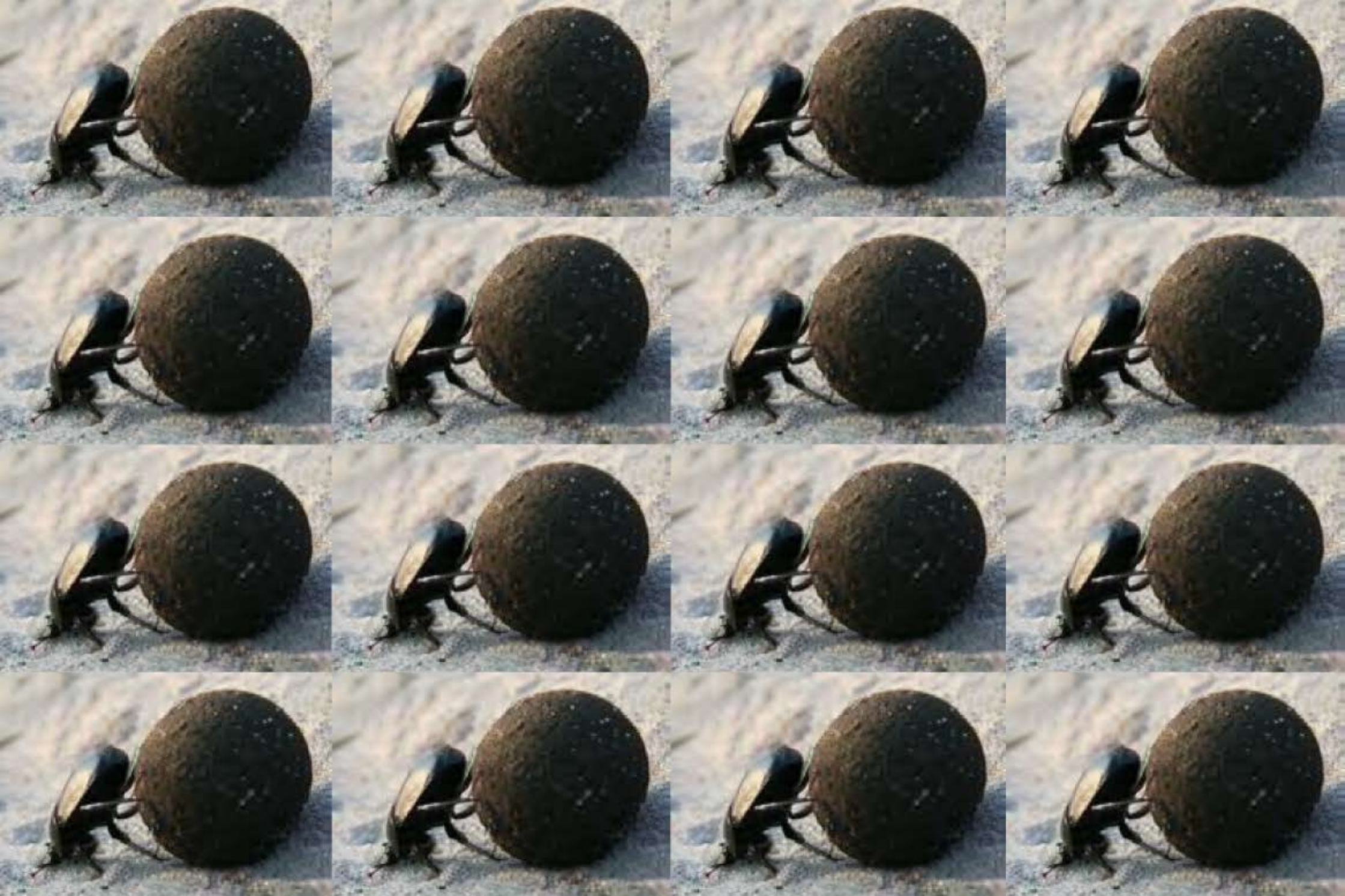


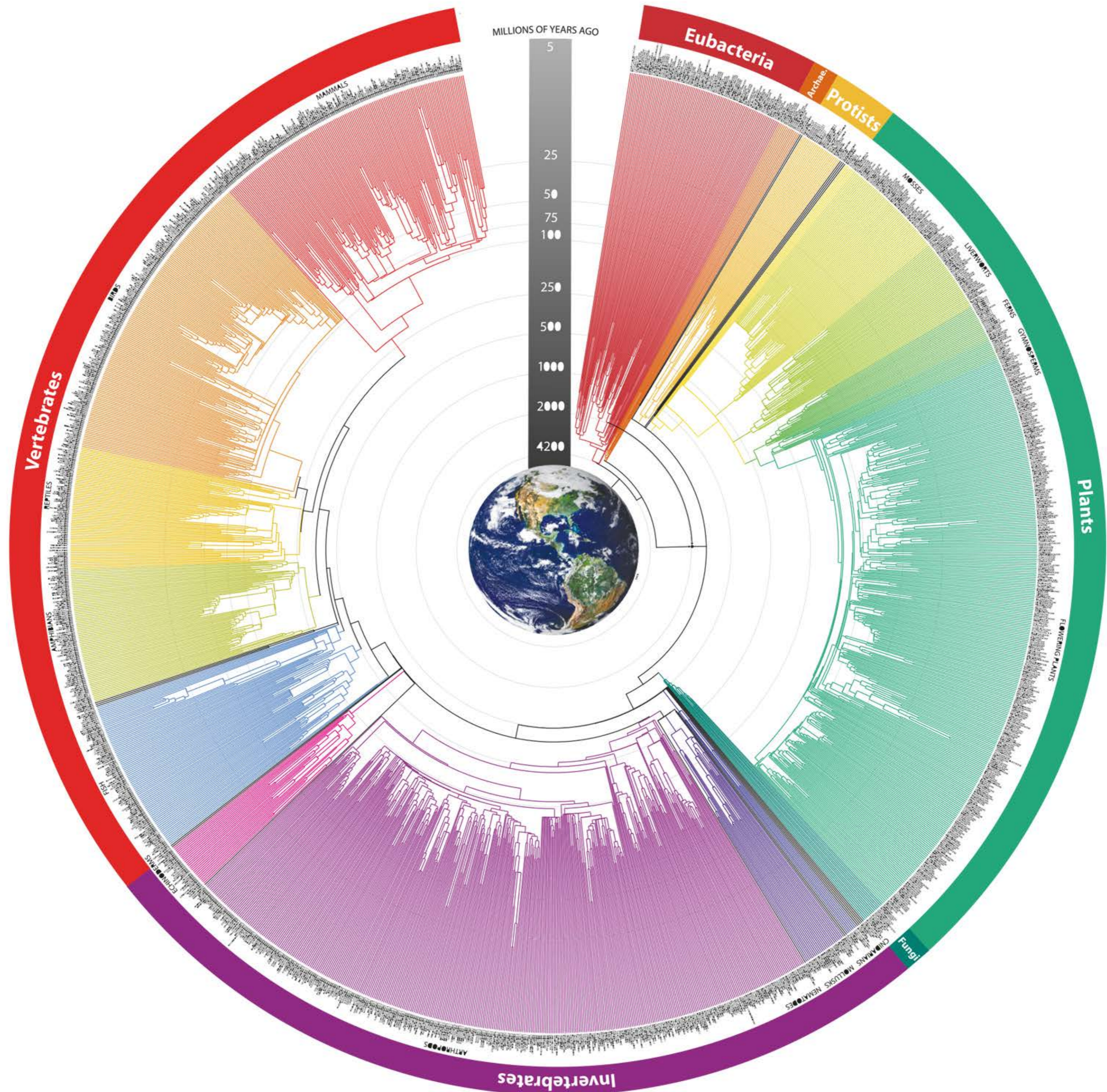




MAN IS BVT A WORM

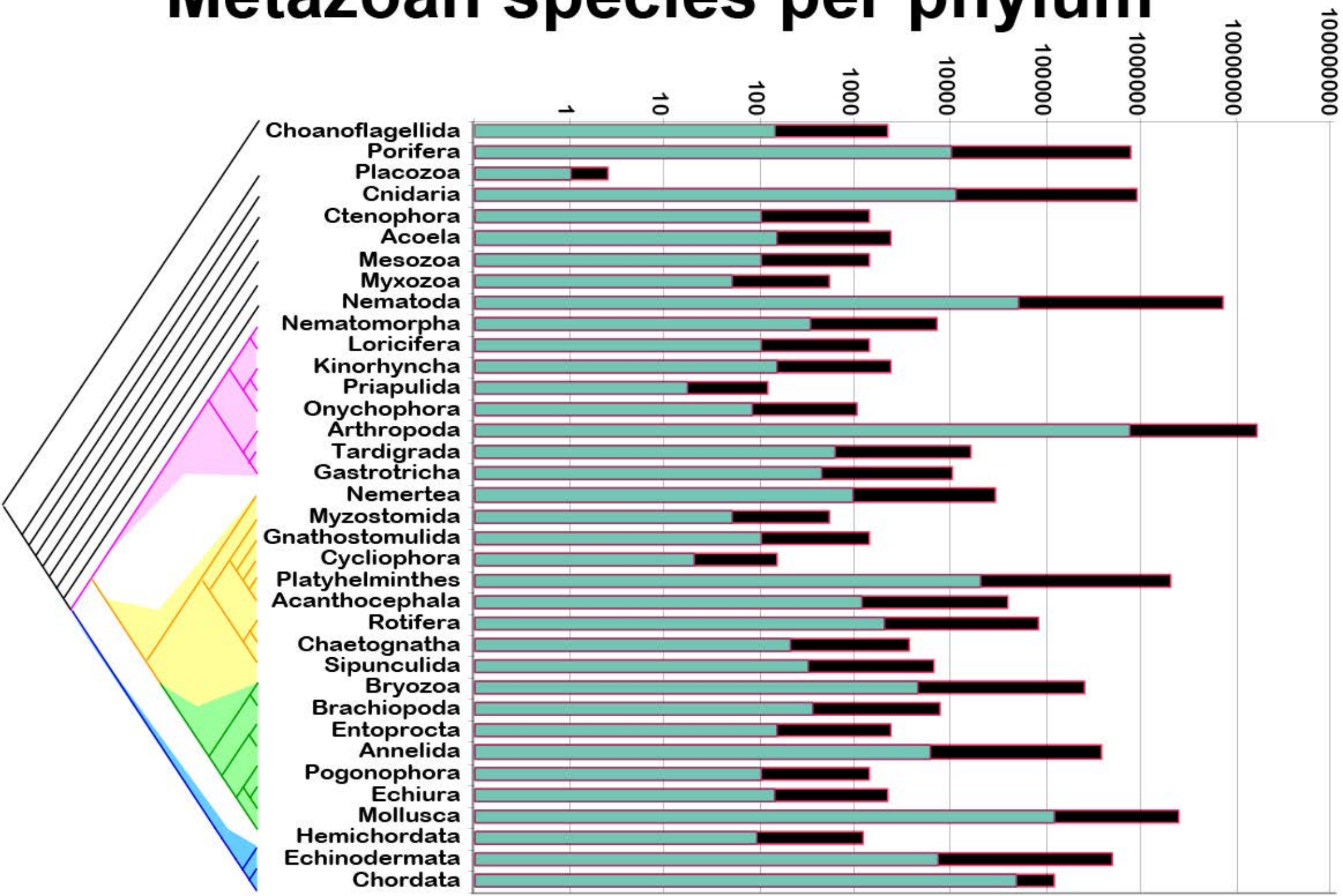


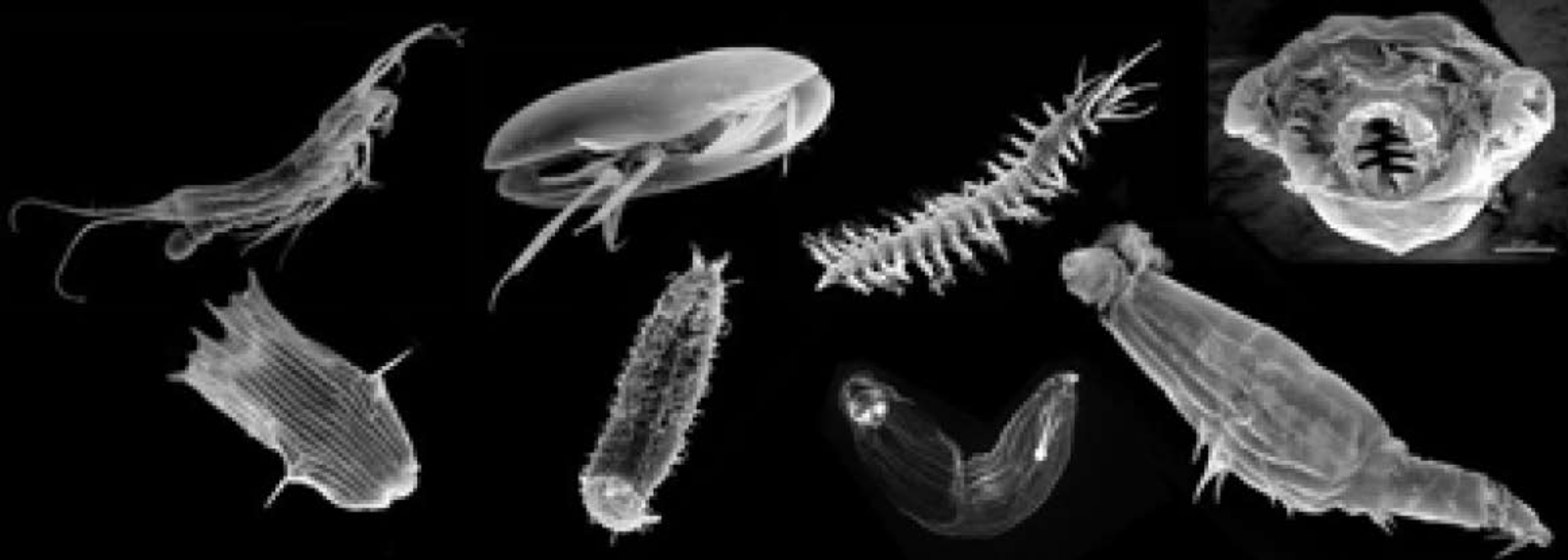




The Timetree of Life (2009) S. Blair Hedges and Sudhir Kumar

Metazoan species per phylum





Clara Flintrop

Zoology Honours undergraduate

James Clark Maxwell Building,
The King's Buildings, Edinburgh

“undisturbed” since 1970



“liverwort”

“moss”



Analysing *meta*DNA barcodes



12-20 million
380-420 base reads
uniformly high phredQ

reject any with N
reject any with $Q < 30$
eliminate phiX174 reads
eliminate too short && too long

all samples	5240308
sample001 (liverwort)	673080
sample002 (moss)	749473
sample003 (liverwort)	863759
sample004 (moss)	1149478
sample005 (liverwort)	938812
sample006 (moss)	865706

Analysing *meta*DNA barcodes



12-20 million
380-420 base reads
uniformly high phredQ

qiime assign taxonomy
RDP classifier
best BLAST match

number of unique OTU @ 98%	25446
unique 98% OTU with >1 in 10e-6 reads	7784
unique 98% OTU with >1 in 10e-5 reads	2366
unique 98% OTU with >1 in 10e-4 reads	521
unique 98% OTU in both sample groups	9174
unique 98% OTU in both, >1 in 10e-4	508
number of 98% OTU ID to named genus	7889
number of 98% OTU ID genus, >1 in 10e-4	222

Phylum	98% MOTU	proportion of total reads
Nematoda	2862	.327
Dikarya	6965	.189
Cercozoa	4254	.085
Annelida	682	.068
<i>null</i>	4867	.057
Streptophyta	614	.057
Oomycetes	487	.056
Bacillariophyta	666	.025
Arthropoda	286	.021
Fungi_incertae_sedis	417	.019
Tardigrada	158	.016
Chytridiomycota	473	.013
Ciliophora	544	.0094
Chlorophyta	473	.0080
Synurophyceae	34	.0059
Centramoebida	288	.0053
Platyhelminthes	94	.0051
Chrysophyceae	198	.0050
Nucleariidae	61	.0048
Tubulinea	194	.0025
Blastocladiomycota	76	.0021
Apicomplexa	74	.0016
Flabellinea	53	.0013
Dinophyceae	139	.0009
Bicosoecida	46	.0007
Uncultured_banisveld_eukaryote	17	.0005
Micronuclearia_podoventralis	20	.0005
Codonosigidae	30	.0005
Ichthyophonida	28	.0004
Px_clade	60	.0004
Fungal_endophyte_sp._sx01	4	.0003
Heterophryidae	27	.0002
Hyphochytriomycetes	9	.0002
Salpingoecidae	13	.0002
Fungal_sp._gmg_c6	37	.0002
Eustigmatophyceae	14	.0001
Capsaspora	10	.0001
Stramenopile_sp._mast-12_kkts_d3	15	.0001
Raphidophyceae	31	.000079
Schizopyrenida	16	.000079
Environmental_samples	8	.000057
Trimastix_pyriformis_atcc50562	5	.000054
Labyrinthulida	16	.000052
Gastrotricha	1	.000041
Telonema	1	.000039
Mollusca	26	.000036
Apusomonadidae	3	.000032
Soil_amoeba_and16	11	.000031
Acanthocystidae	4	.000029
Voromonas	4	.000029
Leukarachnion_sp._atcc_pra-24	1	.000026
Rotifera	10	.000023
Ancyromonadidae	3	.000009
Perkinsea	3	.000004
Mycetozoa	3	.000004
Eccrinaceae	1	.000003
Glomeromycota	2	.000003
Eukaryote_marine_clone_me1-24	1	.000002
Fungal_sp._fcas90	1	.000002
Cryptomonadales	1	.000001
Malawimonas	2	.000001
Phaeothamniophyceae	1	.000001
Chordata	1	.0000003

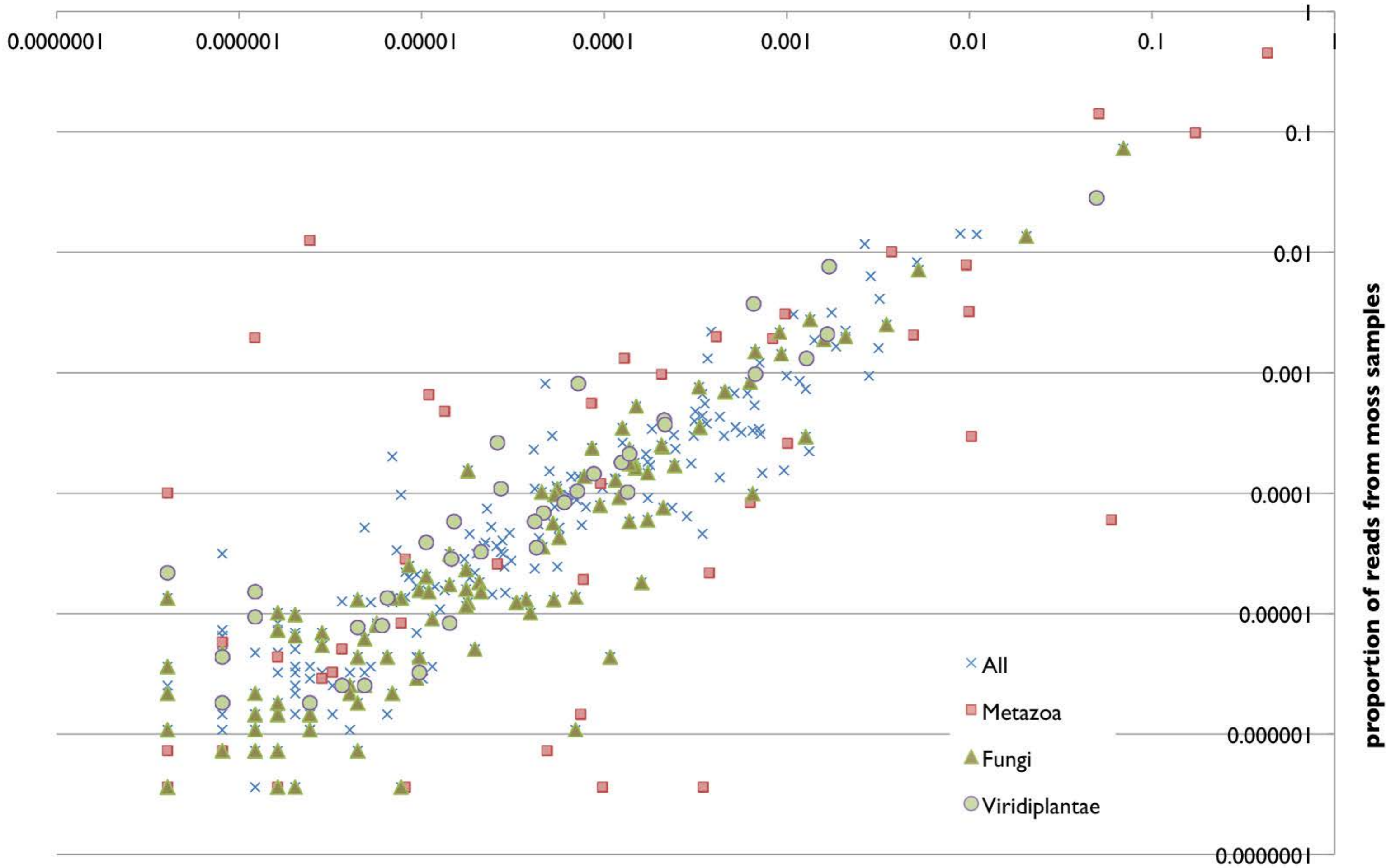
Nine animal phyla represented

Most frequent is **Nematoda**

Most frequent “98% MOTU” is *Plectus (cf aquatilis)*



proportion of reads from liverwort samples

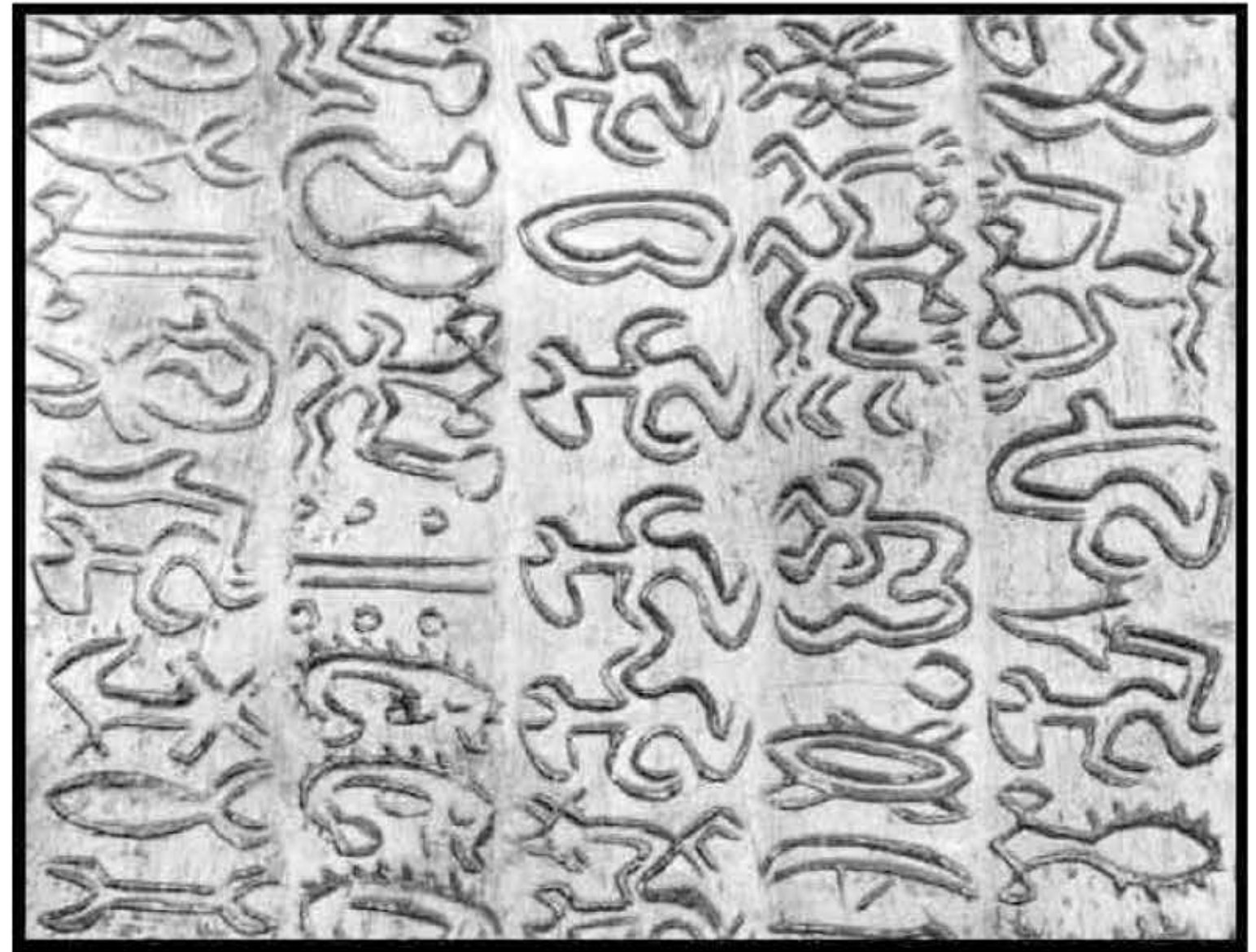


proportion of reads from moss samples

- × All
- Metazoa
- ▲ Fungi
- Viridiplantae

98% uclust OTUs

motu



1. to cut; to snap off

motu-á te hau, the fishing line snapped off

2. to engrave, to inscribe

letters or pictures in stone or in wood, like the *motu mo rogorogo*, inscriptions for recitation in lines called *kohau*.

3. islet

some names of islets: *Motu Motiro Hiva*, *Motu Nui*, *Motu Iti*, *Motu Kaokao*, *Motu Tapu*, *Motu Marotiri*, *Motu Kau*, *Motu Tavake*, *Motu Tautara*, *Motu Ko Hepa Ko Maihori*, *Motu Hava*.

MOTU \approx **BIN** *sensu BOLD Systems*

MOTU \neq **species**

but...

MOTU **often** \sim species

MOTU **often** $==$ cryptic species

multilocus barcoding \approx species

Linnaean taxonomy and “traditional” systematics



Identified specimen-based barcoding
“Platonic” barcoding

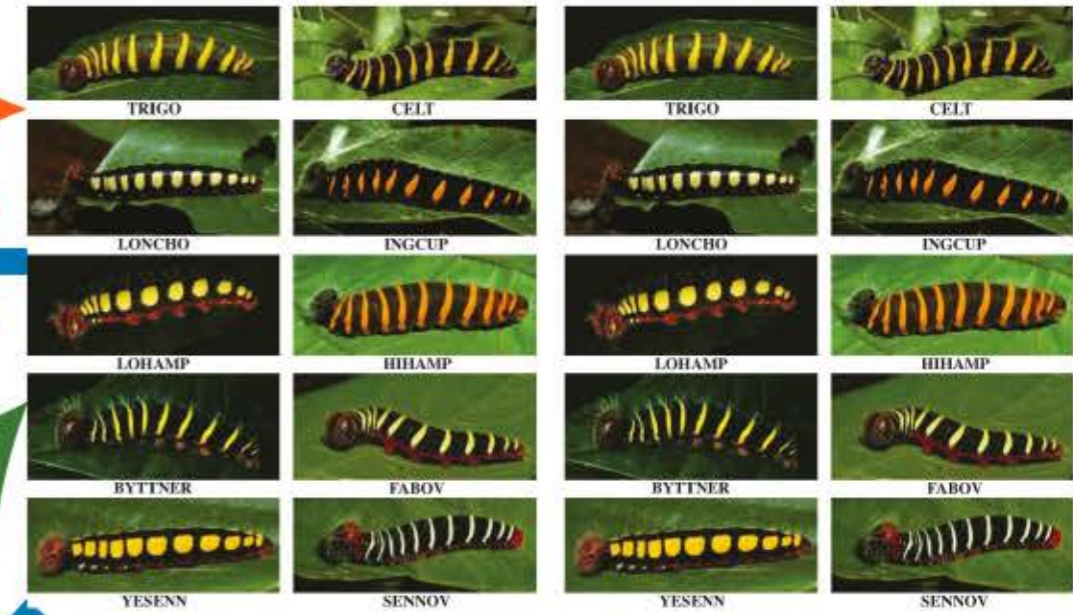
Linnaean taxonomy and "traditional" systematics



Taxonomic Framework

Cryptic taxa
New Taxa

Specimen-based community barcoding



Taxonomic Framework

Additional characters
Cryptic taxa

Reference database
Barcode-specimen links

Improved Reference database

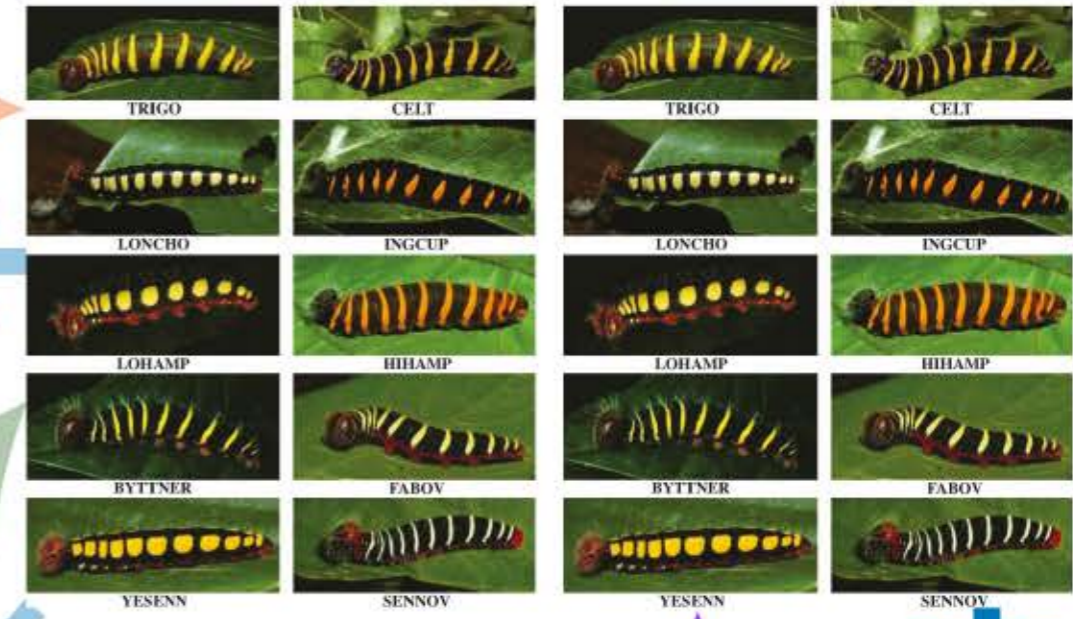


Identified specimen-based barcoding "Platonic" barcoding

Linnaean taxonomy and "traditional" systematics



Specimen-based community barcoding



Identified specimen-based barcoding
"Platonic" barcoding

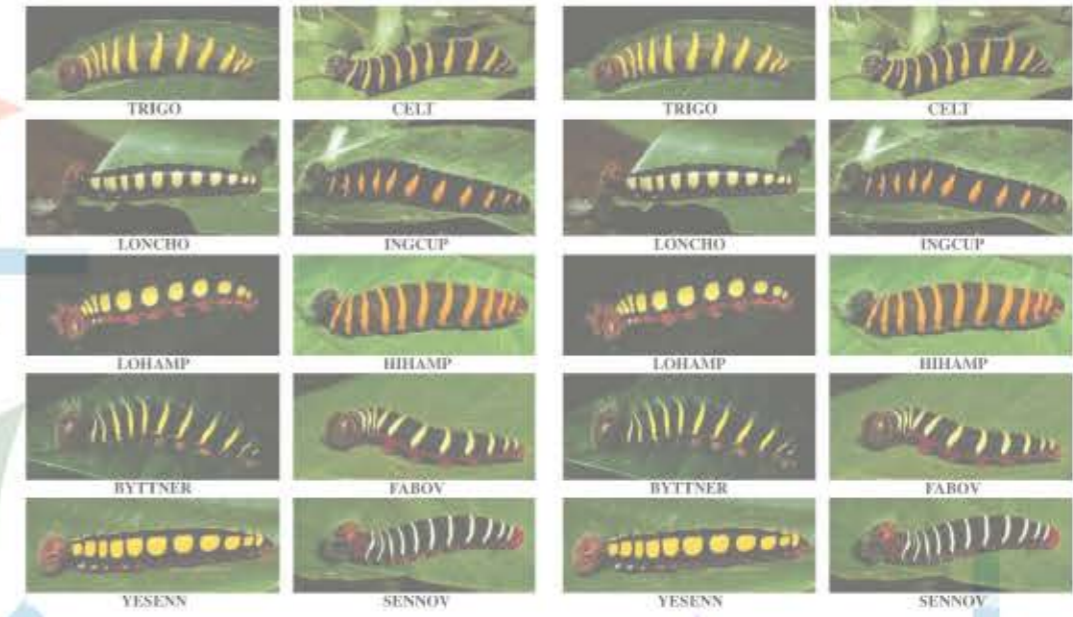
Specimen-independent community barcoding
"Monster Soup" Metabarcoding, Metagenetics

Linnaean taxonomy and "traditional" systematics



Taxonomic Framework

Specimen-based community barcoding



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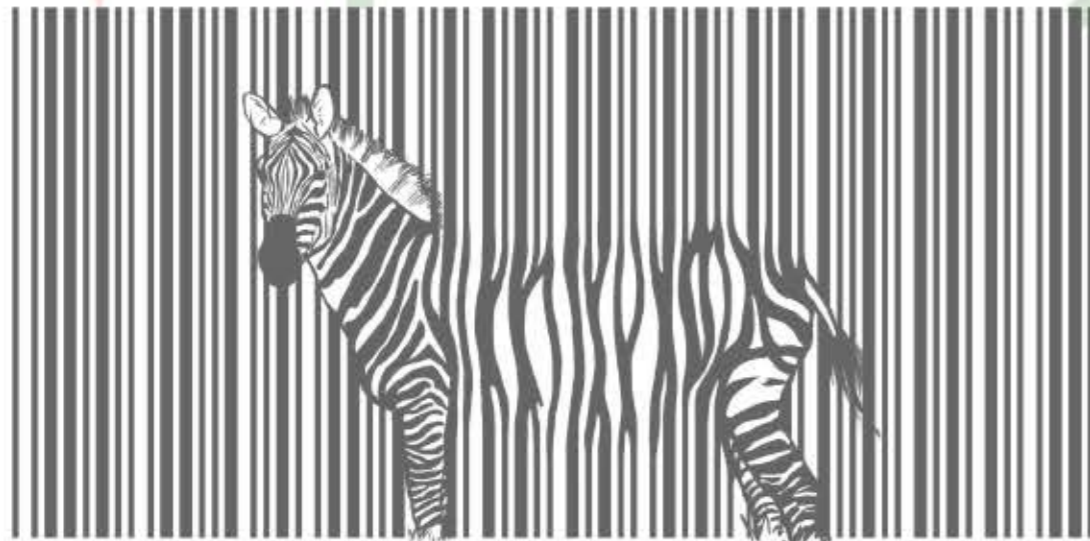
Cryptic taxa
New Taxa

Framework
Taxonomic
Additional characters
Cryptic taxa

Reference database
Barcode-specimen links
Improved Reference
Reference database
Barcode-specimen links

10⁸⁺

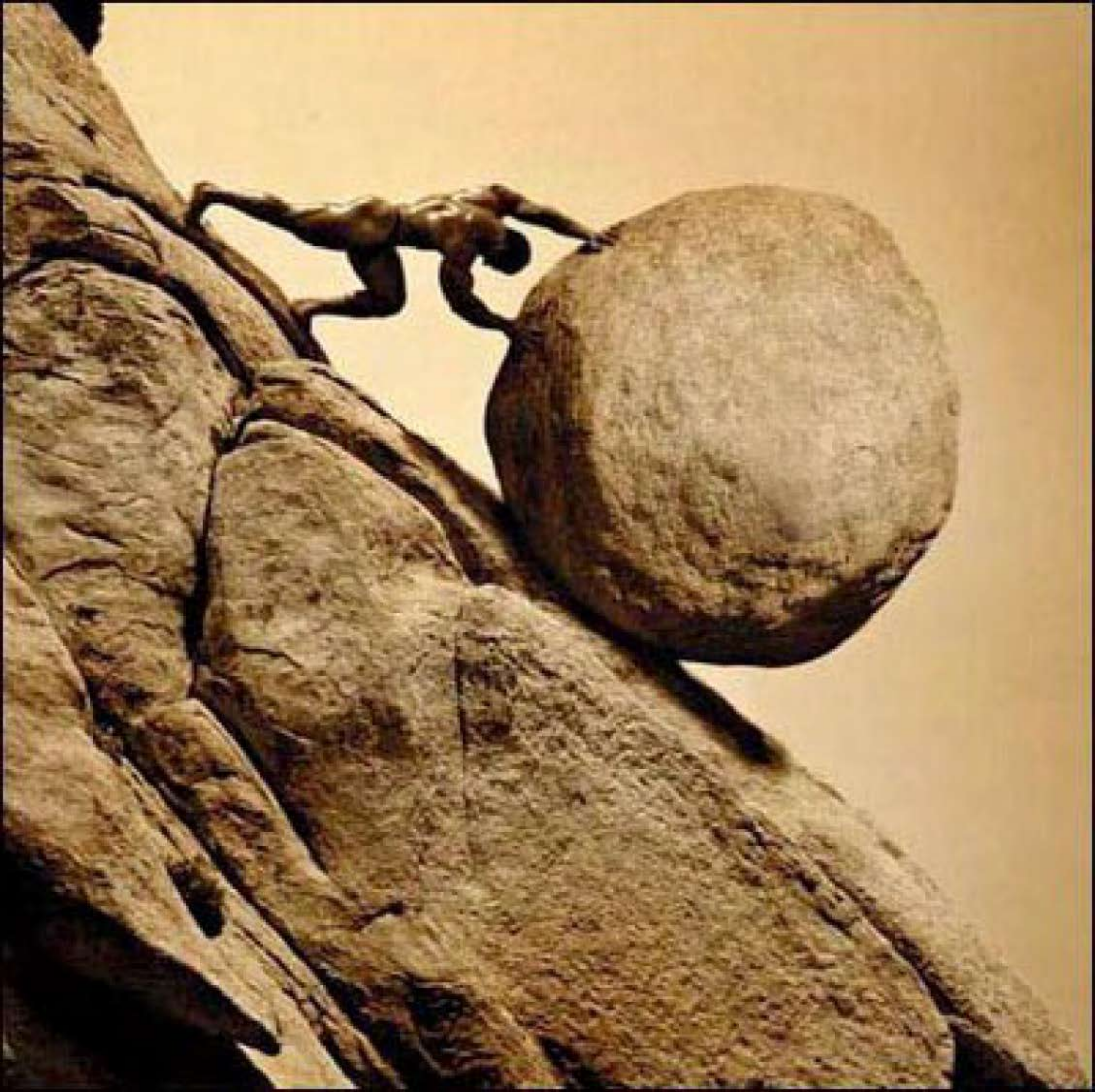
Improved reference
Reference database
Barcode-specimen links



Identified specimen-based barcoding "Platonic" barcoding



Specimen-independent community barcoding "Monster Soup" Metabarcoding, Metagenetics



La lutte elle-même vers les sommets suffit à remplir un coeur d'homme. Il faut imaginer Sisyphe heureux.

Albert Camus 1942

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Martin Jones



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Edinburgh Genomics
(especially Marian Thomson)

Metabarcoding collaborators

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