

Unveiling the hidden: using de novo transcriptomics in barcode analyses and mitogenomic assemblage of unknown Andean velvet worms

Kenny Chen¹, Samin Ragib¹, Genrietta Yagudayeva², Juan C. Santos²

¹Brooklyn Technical High School; ²Department of Biology, St. John's University

Abstract: *Peripatus*, an evolutionarily significant genus of flatworms, are commonly found in low-altitude, humid areas such as tropical or temperate rainforests. Thus, those found in high altitudes like the Andes mountains provide a fascinating line of inquiry into their adaptive molecular evolution. Similar to our previous research, where we identified high-altitude adapted planaria of the genus *Amaga* and reconstructed its mitochondrial (mt) genome, phylogeny, and life history implications, in this project, we present a similar study of high-altitude adapted velvet worms. Using Next-Generation Sequencing (NGS), we TRIzol RNA-extracted, sequenced the transcriptome samples, and isolated the cellular respiration barcode gene, *COX1*, for further analyses. Our methods show a transcriptomic approach in reconstructing the mt-genome and phylogeny of an unknown velvet worm. We suspect that our sample is a potential brother species of Peripatidae's radiative evolutionary family that harbors *Epiperipatus boilleyi* bio-mimetic applications while presenting genetic data of similarly evolved species. Our findings connect with our prior research to holistically explain how these understudied and neglected Andean invertebrates adapt to such high altitudes to explain their adaptation and implications.

Introduction

Recent research on under-studied high-altitude invertebrates in the Andes, such as *Amaga* planaria and *Peripatus* velvet worms have underscored their scientific potential.

- In 2022, our lab identified a high-altitude adapted brother species of *Amaga expatria* [1], noted for its non-chemical pest control potential against crop destroying *Deroceras reticulatum*
- Velvet worms, members of the Onychophora phylum, are diverse in their genera, sizes, colors, and shapes (Fig. 1)
- Velvet worms have been identified globally, illustrating their Gondwana distribution; despite this, their locality is limited to low altitudes with the highest altitude found in the equatorial highlands of Ecuador (Fig. 2)
- Unfortunately, these animals are neglected in their studies compared to Austral relatives [2] and popular neural organismal model *S. mediterranea* [3]
- Velvet worms harbor potential to explain both organismal and planetary evolutionary biology given their ancient nature [4] and radiative Gondwana distribution [5]
- Their slime jet, a defense and prey capture mechanism is applicable in bio-glue and bio-polymer mechanisms [6]
- In 2023, we collected *Peripatus* sp. velvet worm specimens in Cuenca, 2560 meters above sea level and conducted RNA extraction, transcriptome sequencing, and *COX1* barcoding to explore adaptive molecular

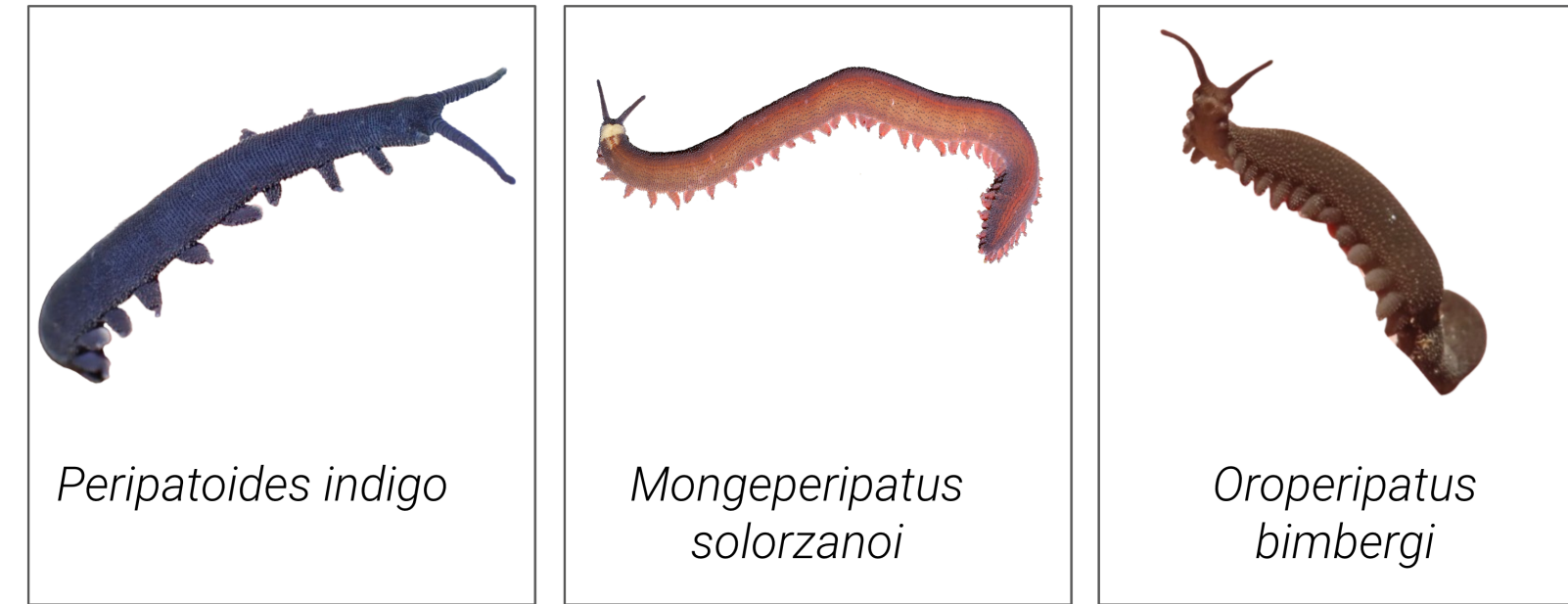


Figure 1: Different species and genera of the Onychophora phylum

- evolution within a six-module method of an RNA-based metagenomic transcriptome assembly approach rather than a DNA-based approach (Fig 3)
- COX1* is the gene that encodes for cytochrome *c* oxidase and is an important discriminator in each species [7]
- The possible high-altitude adaptations of our unknown organism and its applications accompanied by the lack of literature lead elucidate its genetic evolution, contributing to a comprehensive reference database and advancing our understanding of biomedical and industrial applications

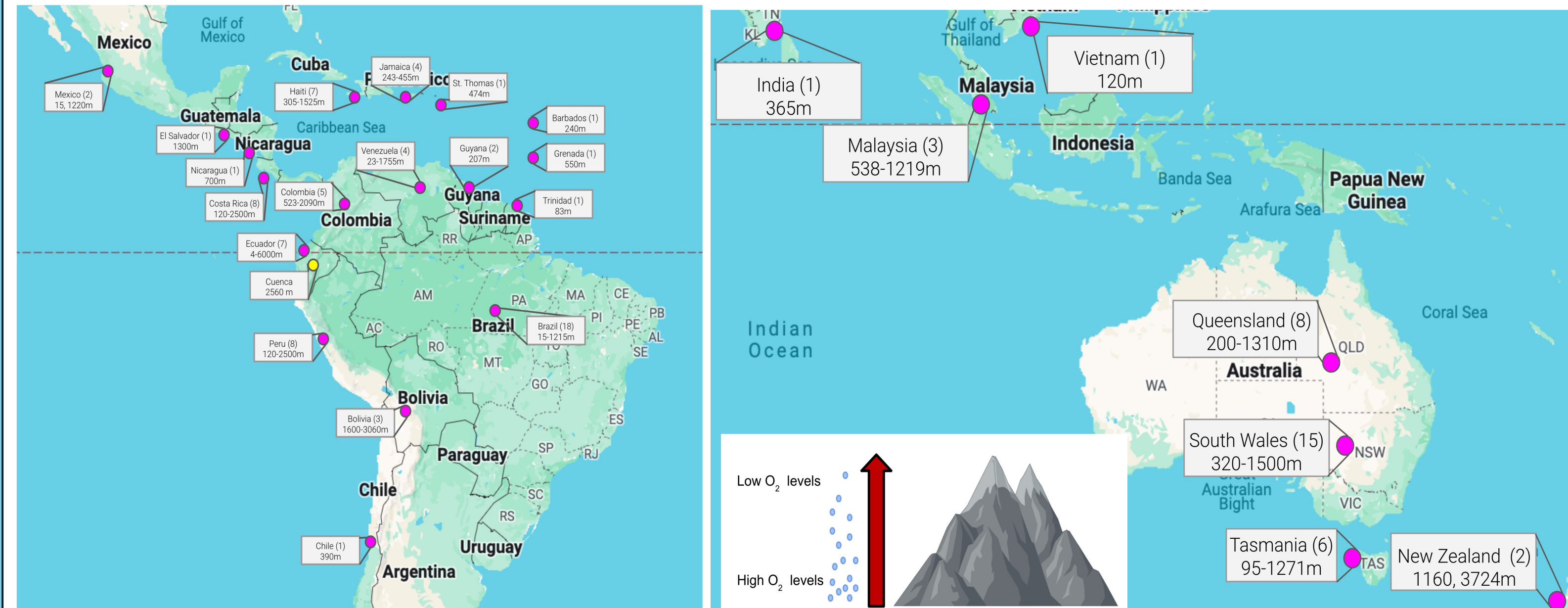


Figure 2: A widespread geographic distribution illustrating the Gondwana distribution of velvet worms (literature locality sightings in purple pins versus our samples in yellow pin) with focus on altitude given that as altitude increases, oxygen concentration decreases, indicating strong potential that selection occurs in high altitude dwelling velvet worms such as our samples

Methods

Total RNA Extraction

TRIZol extraction of RNA. Sent for sequencing, receiving FASTQ files.

Transcriptome Assembly and Annotation

read data from Azenta was fragmented and split into individual genes.

NCBI BLAST

COX1 genes were isolated and genetic relatives collected from BLAST.

Mitogenome Reconstruction

Using MITObim, a reconstruction of the mitogenome was completed.

Phylogenetic Reconstruction

iQTree2 created a phylogeny tree of estimated evolutionary relationships from raw read compared to BLAST sequences.

Slime Jet Analysis

Previous scholarly research was collected on the nature of the *Onychophora* slime jet.

Figure 3: A six-module method of our project involving i) RNA extraction, purification, and sequencing, ii) transcriptome processing via Pincho [8], iii) BLAST analysis, iv) reconstructing mitogenome, v) reconstructing phylogeny, and vi) analyzing the predictions of our sample's slime jet

Results

Velvet worm relatives	BLAST percent identity	e-value	accession number
<i>Epiperipatus boilleyi</i>	78.24%	0	NC_009082.1
<i>Oroperipatus</i> sp. DVL-2011	85.21%	0	NC_015890.1
<i>Epiperipatus acacioi</i>	84.89%	0	PP054361.1
<i>Epiperipatus boilleyi</i>	84.25%	0	HM600781.1
<i>Peripatoides</i> sp. DVL-2010	82.72%	0	HM600782.1

Table 1: The top five relatives of all samples with their percent identity, e-value, and accession number. Also interesting to note, midsections had BLAST outputs of various insects, such as beetles (*Haliphys* or *Monochamus* genera), flies (*Drosophila*, *Lonchoptra*, or *Dicerapanorpa* genera), and mosquitos (*Bironella*, *Anophles*, and *Sabethes*) genera

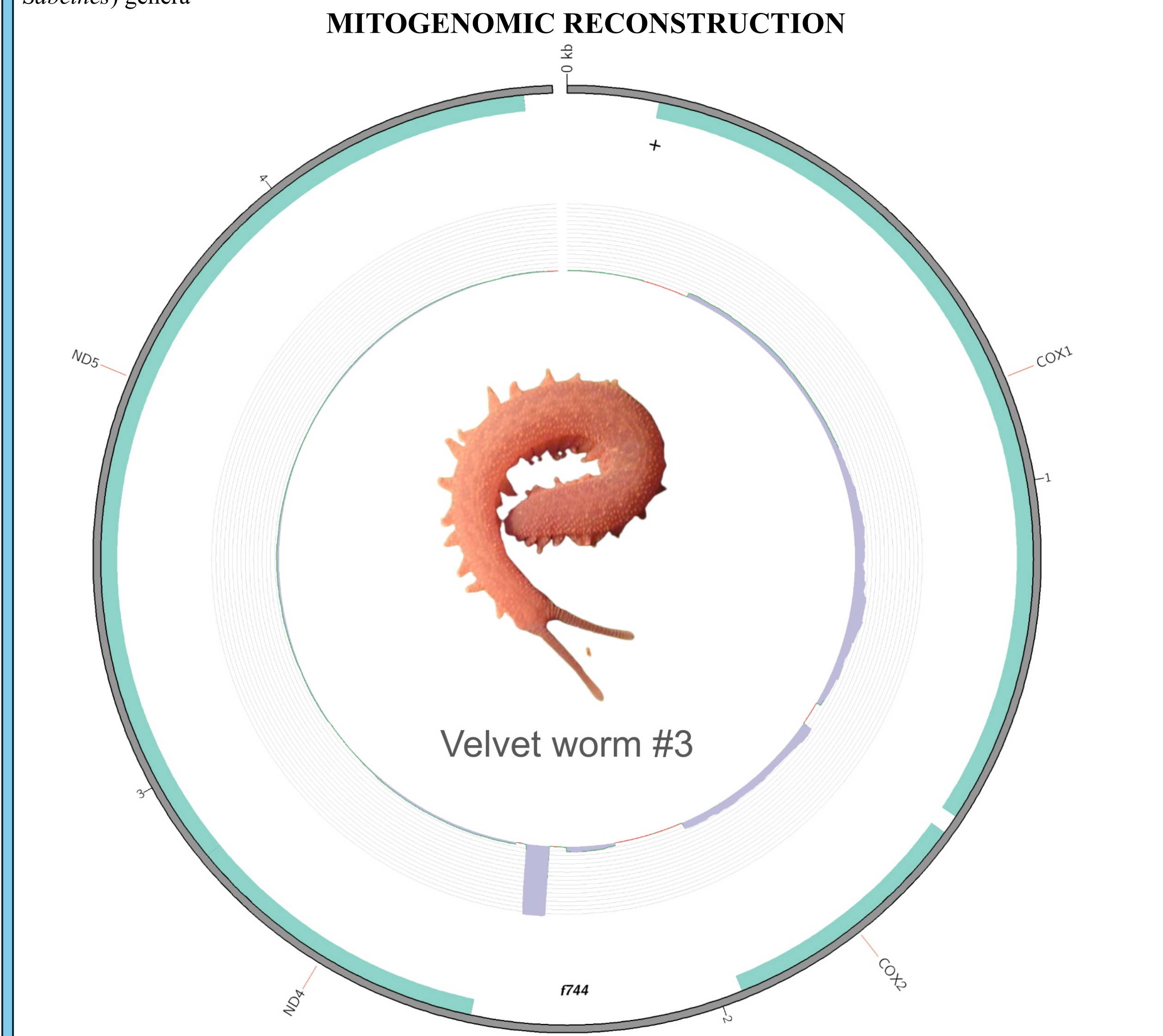


Figure 4: The mitogenome of one of our samples of four genes, *COX1*, *COX2*, *ND4*, and *ND5*, and their respective gene expressions shown by the purple histogram

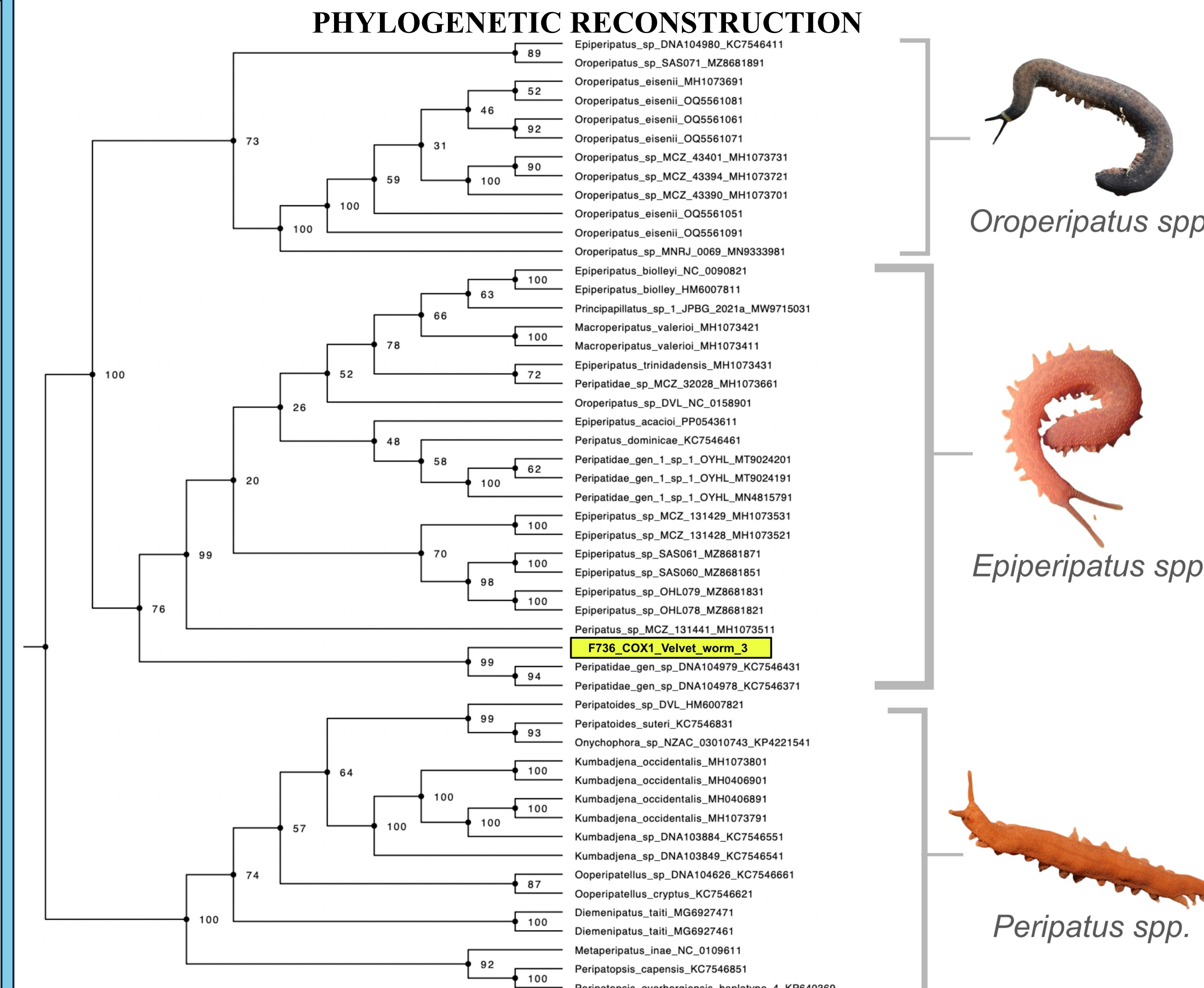


Figure 5: The phylogenetic tree of our sample. The tree is divided into three main clades of genera, and we found that our sample is within the Onychophora phylum, the Peripatidae family, likely of the *Epiperipatus* or *Oroperipatus* genera with bootstrap values representative of each node

Discussion

- Based on **BLAST** (Table 1),
 - our species is confirmed to be a part of the Onychophora phylum, likely of the *Epiperipatus* genus and Peripatidae family, a group of velvet worms inhabit the neotropics and is found in high altitudes and viviparous
 - given the unresolved phylogenetic relationships between velvet worms and the evolutionary radiation of the Gondwana distribution, our results show precise phylogenetic distinctions via transcriptomics
 - various insects within the midsections of the specimen were identified, indicating the sample's carnivorous diet via the precision of transcriptomics
- Based on our **mitogenomic reconstruction** (Fig. 4),
 - we were able to harvest four mitogenes from our sample; it was anticipated to be incomplete given the lack of genetic data available on these animals
 - it is necessary to develop more refined methods in mitogenomics, such as transcriptomics
- Based on our **phylogenetic reconstruction** (Fig. 5),
 - the animal likely of the high-altitude adapted genus *Epiperipatus*, explaining how these cryptic species evolved and what it means to speciation and the evolution of our Earth, often misunderstood from sole morphological analysis
- Based on our **slime jet inference** (Fig. 6),
 - slime jet frequency and hardening time after making contact with the surface can be made
 - findings imply a new relative species in bio-adhesive and biopolymer applications with potential in human wound and organ care without the harm of artificially synthesized polymer additives

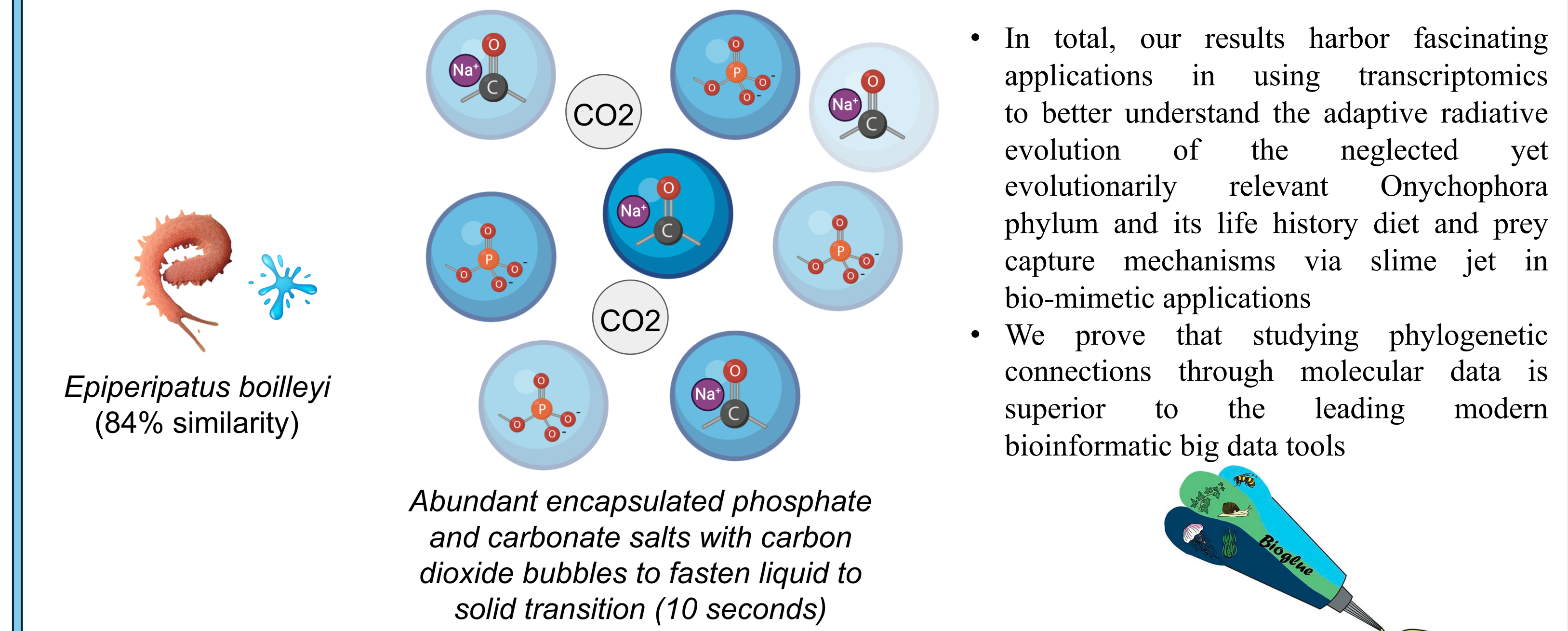


Figure 6: Predictions to be made given our sample's 84% similarity to the applicable *E. boilleyi*; potential in bio-polymerization and bio-glues

Future Steps

- Investigate more velvet worms and Andean invertebrates to grow the scope and sample size to increase the transcriptomic data available of velvet worms
- Perform differential gene expression analysis to understand the genetic differences between highland and lowland living velvet worms by analyzing adaptation-specific mutations
- Continue research on the velvet worm slime jets as they have the potential to play a crucial role in the field of medicine and industrial design; in the future, we may find other similarly evolved species with these similar mechanisms to expand current databases (e.g. other worms, snails, frogs, fish, etc.)

WBM Type: TTAATGACTCTCCGATGGTAACTCTG
 Mutant Type: TTAATGACTCTCCGATGGTAACTCTGAA

Figure 7: Sequence analysis potential between velvet worms based on localities



Figure 8: Slime jet cannon release of *Principillatus hitoyensis*

References

- Justine, Jean-Lou, et al. "The land flatworm *Amaga expatria* (Geoplanidae) in Guadeloupe and Martinique: new reports and molecular characterization including complete mitogenome." *PeerJ*, 9 November 2020. DOI: 10.7717/
- Oliveira, I. S., Lacorte, G. A., Fonseca, C. G., Wieloch, A. H., & Mayer, G. (2011). Cryptic speciation in Brazilian *Peripatus* (Onychophora: Peripatidae) reveals an underestimated diversity among the peripatid velvet worms. *Plos one*, 6(6), e19973.
- Grohme, M. A., Schloissig, S., Rozanski, A., Pippel, M., Young, G. R., Winkler, S., Brandl, H., Henry, I., Dahl, A., Powell, S., Hiller, M., Myers, E., & Rink, J. C. (2018). The genome of *Schmidtea mediterranea* and the evolution of core cellular mechanisms. *Nature*, 554(7690), 56–61. <https://doi.org/10.1038/nature25473>
- Peripatus (Onychophora). (n.d.). Manaaki Whenua. Retrieved May 15, 2024, from <https://www.landcareresearch.co.nz/discover-our-research/biodiversity-biosecurity/plants-invertebrates-fungi-and-bacteria/invertebrate-systematics/peripatus/>
- Baker, C. M., Buckman-Young, R. S., Costa, C. S., & Giribet, G. (2021). Phylogenomic analysis of velvet worms (Onychophora) uncovers an evolutionary radiation in the neotropics. *Molecular Biology and Evolution*, 38(12), 5391–5404. <https://doi.org/10.1093/molbev/msab318>
- Corrales-Ureña et al. (2022). Encapsulated salts in velvet worm slime drive its hardening. *Scientific Reports*, 12(1), 19261. <https://doi.org/10.1038/s41598-022-23523-2>
- Strüder-Kypke, et al. "Comparative analysis of the mitochondrial cytochrome c subunit I (COI) gene in ciliates (Alveolata, Ciliophora) and evaluation of its suitability as a biodiversity marker." *Systematics and Biodiversity*, 8, 1, 131–148, DOI: 10.1080
- Ortiz, Randy, et al. "Pincho: A Modular Approach to High Quality De Novo Transcriptomics." *Genes* 12, 22 June 2021, no. 7: 953, DOI: 10.3390

Acknowledgements

This project was possible due to the generous support from our mentor, Genrietta Yagudayeva and the Principal Investigator, Dr. Juan C. Santos. We thank Allison Mayle and the Urban Barcode Research Program (UBRP) for guidance, and the Pinkerton Foundation and Science Sandbox for generous financial support.

