

Assemblage of the mitochondrial genome of an unknown planarian species from the Andes and its phylogenetic comparison with temperate and lowland relatives

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Abstract

Using Next Generation Sequencing (NGS), an unknown planaria species, collected in Ecuador, underwent RNA extraction, and its transcriptome was sequenced. Using the BLAST tool in the NCBI database, a part of the mt-genome of an unknown planarian was compared to all the registered mt-genomes in the database to see which organism it was related to the closest. We found that the sample corresponded to an unknown species of Amaga, which is widespread in South America. Interestingly, we found that one of the individuals sequenced had sequences of a possible prey item, a common invasive milky slug (*Deroceras reticulatum*): a European pest found worldwide. Our results provide the first evidence of predation of planaria on milky slugs and suggest possible means of pest control. This project aims to further the genetic database of living organisms, specifically genetic data on planarians.

Introduction

In this project, we plan to sequence the entire mt-genome of an unknown planarian species from the Andes of Ecuador. The mt-genome, with its small size, easy sequencing, and clear language, is an accessible way to specify components of DNA within the mitochondrion, which contains genes critical for cellular respiration¹. We will use the COX1 barcode of this species and compare it with other planarians available in the Andes region. We chose the COX1 mitochondrial gene as it has been proven highly efficient for genetic analysis for invertebrates and vertebrates². For our proposal, we will focus on a planarian, a flatworm within the Rhabditophora group. Unfortunately, not much is known about the evolutionary history of planarians. There are barely any available fossil records of flatworms, so most information and theories need to be drawn from amber fossils, coprolites, estimates of mutation rates, and paleogeographical information³. For this project, we will reconstruct the mt-genome of an unknown Andes-dwelling planarian species from transcriptomic data⁴. To sequence the transcriptomes, we will use next generation sequencing (NGS), which will determine only a slight portion of the nucleotide sequence of an organism's genome. With the help of DNA sequencing technologies, multiple DNA sequences in parallel are processed. For this project, we used RNAseq of an unknown Andean planarian and isolated its mitochondrial genome. There are very few studies in which researchers have used RNAseq to isolate the mt-genome of planarians. Therefore, our project would be the first attempt to isolate an entire mt-genome for this unknown species. We found that our sample corresponds to an unknown species of Amaga and evidence of possible predation by this species on the invasive milky slug (*Deroceras reticulatum*). This project aims to further the genetic database of living organisms, specifically genetic data on planarians.

Methods & Materials

<p>Total RNA Extraction</p> <p>Extract & Check Purity of RNA.</p> <p>Sent Results to Genewiz (Azenta). sequenced the entire transcriptome.</p> <p>Results returned in raw files without any modifications with forward and backward read files for each file.</p>	<p>Transcriptome Assembly & Annotation</p> <p>Used <i>Pincho</i> to assemble transcriptome (UniProt).</p> <p>Around 3000 bp of the mt-genome was used in BLAST in the NCBI database to identify the species/closely related species.</p> <p>Output revealed some of the identity of the unknown planaria as an Amaga sp. and likely prey, the invasive milky slug (<i>Deroceras reticulatum</i>).</p>	<p>Planaria mt-genome Isolation</p> <p>Entire genome was unable to be reconstructed thus additional software (minimap2 and SAM-tools) were required.</p> <p>Minimap and SAM-tools successfully aligned and manipulated the data to the reference Amaga expatria species.</p> <p>Finally the mt-genome was reconstructed with MITObim software (standard parameters).</p>	<p>Planaria & Milky Slug Phylogeny</p> <p>With the extracted <i>Deroceras</i> and Amaga COX1 genes the 20 closely related species were chosen for phylogenetic comparison.</p> <p>Alignments were made using the R packages DECIPHER. Maximum likelihood tree was estimated using IQ-TREE2.</p> <p>Determine bootstrap support of the phylogenies with 1000 non parametric bootstrap repeats.</p>
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Results

Mitochondrial Genome Results

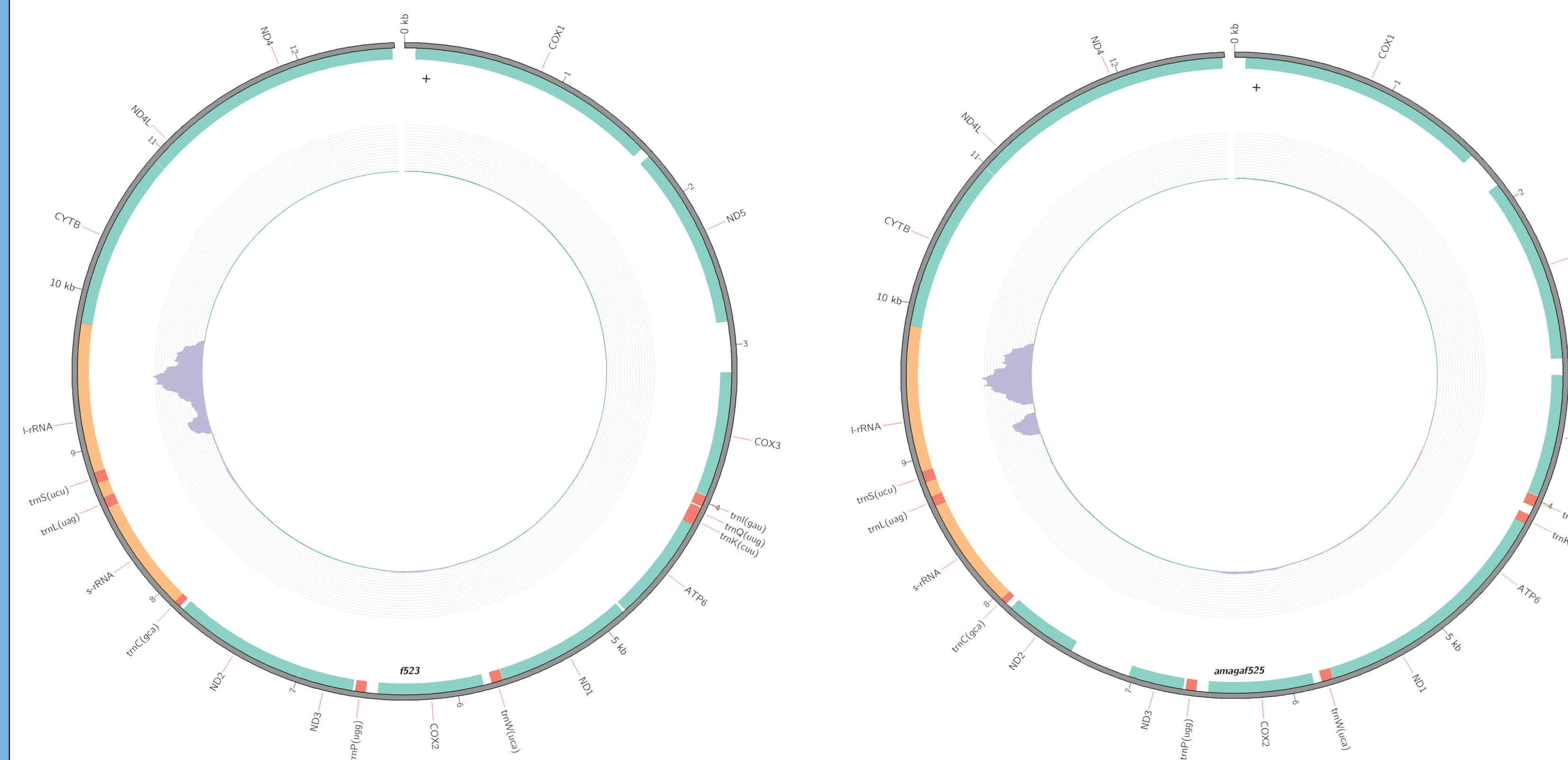


Fig 1. Amaga sp. mtDNA genome of two samples: F523, F525. We extracted the COX1 for barcode analyses.

Phylogenetic Tree Results

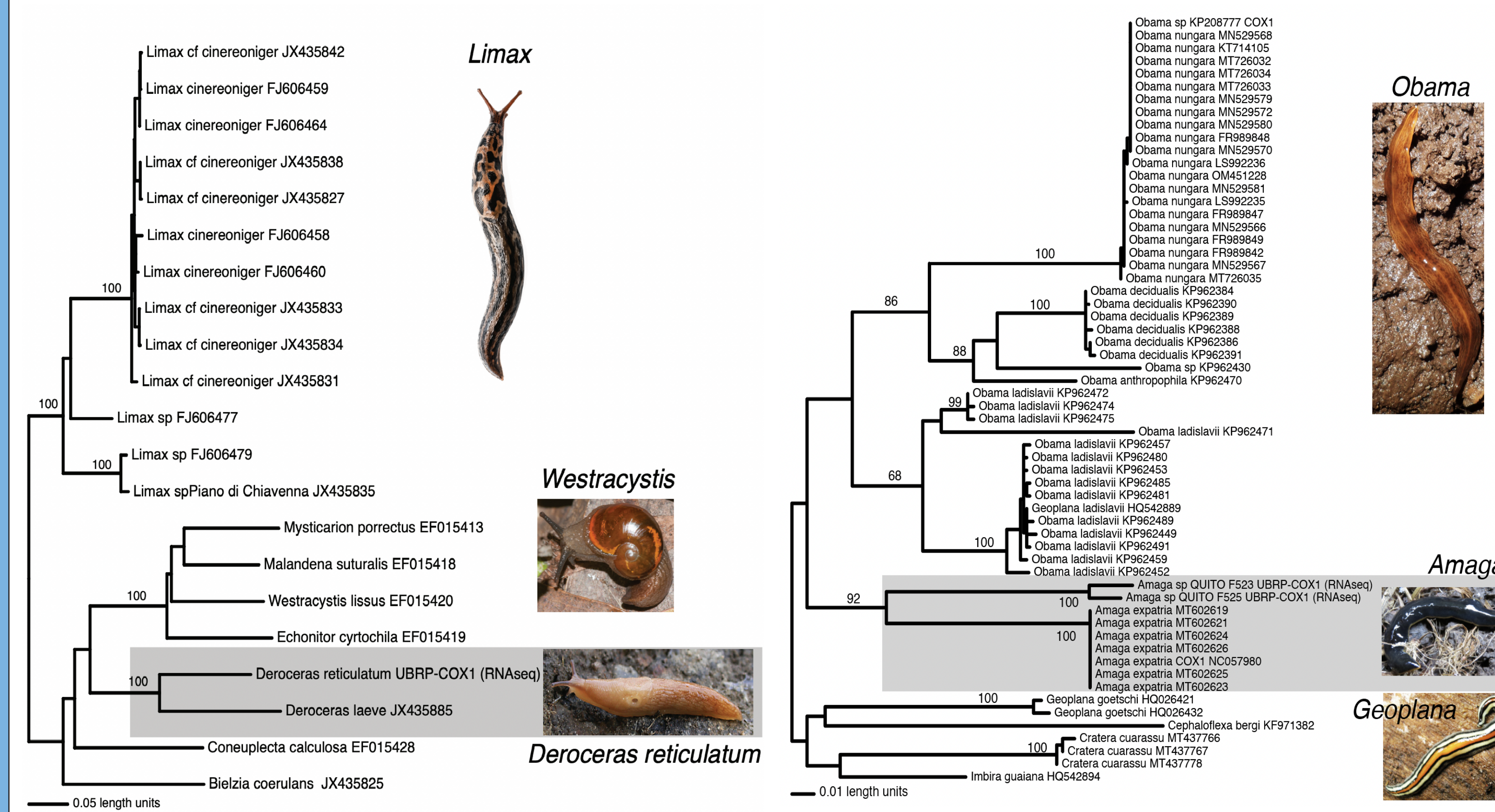


Fig 2. Amaga sp. phylogeny, the nodal support with bootstraps >75 are indicated.

Fig 3. Deroceras reticulatum phylogeny, the nodal support with bootstraps >75 are indicated. The mtDNA genome of the slug was isolated from RNAseq reads for Amaga.

Discussion

As a whole, three samples of the planaria were extracted, sent to Azenta for sequencing, and then had the mt-genome reconstructions (Fig. 1). From BLAST, we were able to find that two of the samples were actually from a common garden slug found in Ecuador. The third sample, however, was a planaria specie closely related to the species Amaga expatria with an 89% similarity rate and with the closest e-value. As we found that the sample was only 89% related to Amaga expatria, we know that it is not actually that species but somewhat related. This means that this planaria specie that was found in Ecuador is a new undiscovered species or a close brother species. The slugs that were found in BLAST instead of the planaria indicate that they were eaten and thus were sequenced. This shows the sheer power of transcriptome technology as it was even able to reach the prey of the planaria which was sequenced as the mt-genome. The closest relative with COX1 information to our Amaga species is Amaga expatria. Our phylogenetic tree for Amaga supported its close association (Fig. 2). In recent history, Amaga expatria has been found as an alien species in Guadeloupe and Martinique, France. These planarians can have a carnivorous diet which answers the question of the slugs. In total, there have been only 20 reported sightings of the planaria in pictures and 7 total specimens. An interesting result we found is evidence of a predatory event of the unknown Amaga on the invasive slug, *Deroceras reticulatum*. Our phylogenetic tree for *Deroceras* supported a nearly identical sequence for COX1 for *Deroceras reticulatum*.

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