Classification of unknown deep-sea snailfishes through morphological and genetic evidence

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Abstract

With the high diversity of life on Earth, new species are constantly being discovered. Every species goes through a taxonomic classification process to determine its place in the tree of life. Taxonomy involves examining the morphology of each species and describing its features. However, multiple species can share morphological characteristics, making it difficult to distinguish one species from another based on visual clues alone. Therefore, genetic data can provide powerful insights into relationships between species. Snailfishes (Family Liparidae) live in cold and temperate ocean waters from the intertidal zone to the deep sea. Snailfishes share morphological characteristics including scaleless, tadpole-like bodies and commonly a ventral suction disk, yet they can vary by environment. In this study, we focus on three deep-sea snailfishes that were caught in the Eastern Pacific Ocean, off California. Using micro-CT (microcomputed tomography) scans and physical measurements, we compared characteristics such as body ratios and fin ray counts. We then sequenced barcode genes to position these species in a phylogenetic context. By comparing the morphological and genetic data from our unknown snailfish species to those known, we can discover if they indeed constitute new species, furthering our understanding of the vast biodiversity in our oceans.

Methods

Collection of Fish

Table 1: This table represents where all of the snailfish were found in the world's oceans and the depths they were collected.

*Station M is one of the the most detailed investigations of any abyssal area in the world's oceans. It is located 200 km west of the central California coast (34º 50'N, 123º 00'W; depth of 4000 meters). The seafloor at Station M is characterized by silty-clay sediments.

Unidentified Snailfish Species	Depth of Capture (m)	Location Captured
Pale Snailfish	3,500	Eastern Pacific
Small Black Snailfish	4,000	Station M*
Large Black Snailfish	4,000	Station M*

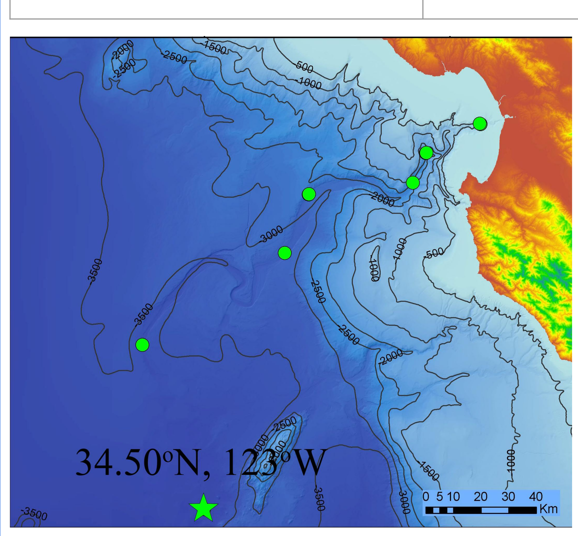

Figure 1.1: This is a visual representation of the location of Station M in the world represented by the green star. These coordinates lie SSW of Monterey Bay (where the the furthest right green dot lies) and WNW of Los Angeles.

Preservation- The snailfish were first preserved in 4% formaldehyde, then transferred to 75% ethanol to prevent the organism for decaying and to preserve its tissues.

Counting and Measuring-

SlicerMorph: SlicerMorph is a computer program that acts as a toolkit to measure, manipulate, and visualize images, specifically CT data. We used this program to visualize and measure features on the snailfishes that are not capable by hand (e.g. otoliths, pharyngeal jaws, and fin ray counts)

CT Vox: This computer program was utilized in visualizing and cutting out unnecessary pieces of CT data of the snailfish so it can be imported to SlicerMorph for precise measurements and close-up pictures of internal and external pictures.

Physical Measurements: We took physical measurements of the snailfish using calipers. The calipers were used to measure distances (e.g. head length/width, eye width, snout length, etc).

Taxonomic Analysis

Figure 2.1: Large Black Snailfish

Figure 2.2: Small Black Snailfish

Figure 2.3: Pale Snailfish

Figure 3: CT scan of ventral suction disk of Pale Snailfish. Disk function is for suction onto rocks.

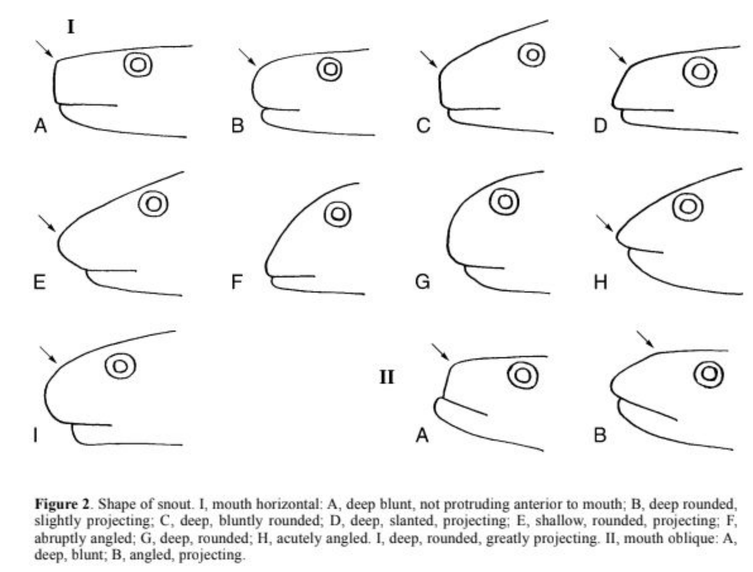

Figure 4: Snout shapes of snailfish (Stein *et. al.*, 2001)

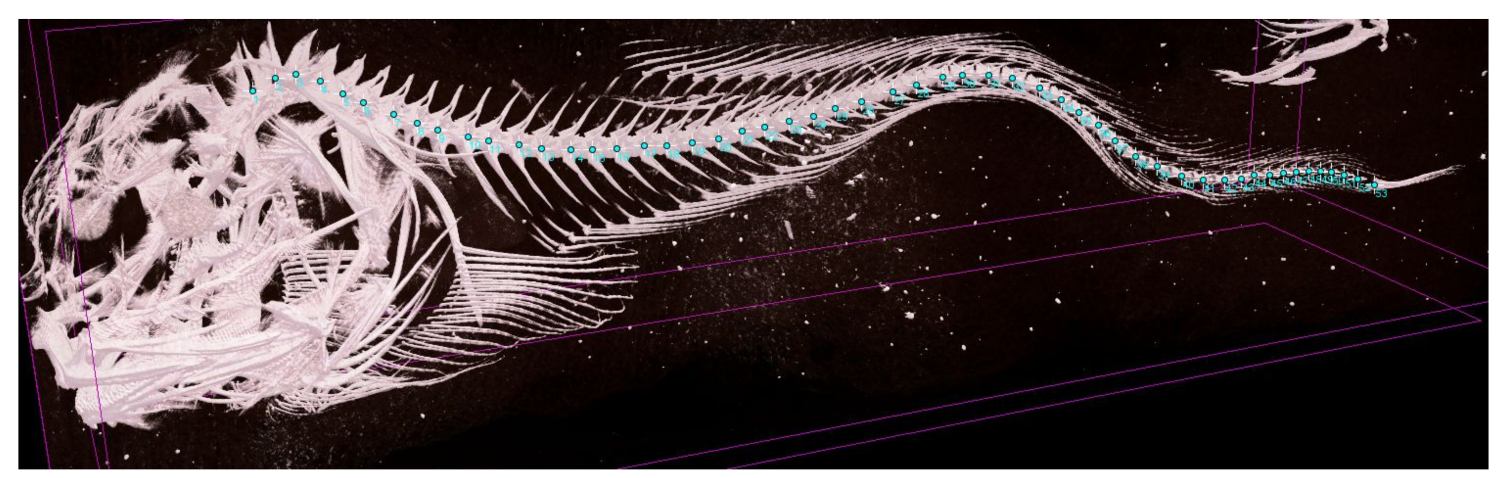

Figure 5: Full CT scan of Pale Snailfish. Counts of vertebrae and rays were based off of CT scan

Table 2: Physical measurements and counts of three unknown snailfishes.

	Pale Snailfish	Large Black Snailfish	Small Black Snailfish
Vertebrae: (Total, abdominal, caudal)	53 (9+44)	63 (11+52)	85 (11+74)
Pectoral Fin Rays (Total, Upper, Notch, Lower):	22 (15+4+3)	22 (13+4+5)	17 (10+3+4)
Caudal Fin Rays (Total, upper, lower):	8 (4+4)	10 (5+5)	4 (3+1)
Branchiostegal Fin Rays:	6	6	5
Dorsal Fin Rays:	43	57	41
Anal Fin Rays:	40	46	N/A
Head Description (refer to Figure 2)	Head, round Snout, deep rounded Nostrils, large, protruding, tubular Mouth horizontal, moderately large, subterminal	Head shape rounded Snout deep, bluntly rounded Nostrils single Mouth is horizontal	Head shape laterally compressed Snout abruptly angled Nostrils are single Mouth slightly oblique, large
% Standard Length Measurements Disk to anus	4.2 %SL	8.9 %SL	N/A
Snout to Anus	24.1 %SL	23.5 %SL	17.1 %SL
Anus to anal Fin	21.9 %SL	19.6 %SL	8.3 %SL
Total Length of Fish (mm)	92.2	174.0	113.1
Ventral Disk	Present	Present	Not present

Results

Based on physical observations, the pale snailfish appears to be a different species from the other two based on the dramatic difference in color, which likely indicates a separate species.

We believe the small black snailfish is a different species from the pale snailfish and the large black snailfish due to the pale and large black snailfish having a ventral suction disk present, whereas the small black snailfish does not. It is very common in snailfish to see disks, so not seeing one in the small black snailfish likely indicates different species.

Snout shape and size are also an indicators of different species. All three fish are characterized by different snout shapes and head shapes, which indicates they might be different species (Stein *et. al.*, 2001).

Each fish also contains different vertebral counts and ray counts (Table 3). The differences in % Standard Length of the snailfish also indicates that the small black snailfish is different from the other two because the anus is in the posterior portion of the body, where the other two fish have the anus in the anterior portion of the body.

Together, our observations suggest that these three fish are not the same species and do not match known species.

Conclusion

Based on our analyses, we believe the three snailfish samples are different species. Physiological observations of the specimens include ray counts, vertebrae, and % standard length measurements. Presence of a disk also was important in our conclusions. Future work analyzing mitochondrial DNA (*COI*, *Cyt-b*, *16S* genes) to test our conclusions based on physiological traits. This will also allow us to confirm our hypothesis that all three snailfish specimens are new species of snailfish based on our comparisons with known species. In the future, we will make phylogenetic trees using the DNA sequences to add to the tree of life of snailfish species.

References

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