Oopsacas minuta, an Amoebozoan Animal:

Genetic Evidence Supports a Case of Multicellular Evolution from Amoebozoan Ancestry

Authors: Ashley M. Gjøvik, B.S., J.D. (ashleymgjovik@protonmail.com) | **Date**: July 06 2025

Abstract

Comprehensive molecular analysis of Lyssacinosida glass sponge *Oopsacas minuta* with bidirectional BLAST searches produced long-alignment, 80–85% sequence identity matches with nucleariid amoebozoans (*Nuclearia thermophila*, *N. simplex*, *N. pattersoni*) across 18S rRNA, 28S rRNA, and mitochondrial markers. Cross validation reveals strong affinities to *Fonticula alba*, including across a 5.2 kb pre-mRNA processing factor alignment. Recursive BLAST analysis revealed additional strong matches between *Oopsacas minuta* and Amoebozoa, predatory fungi, complex protists, Excavata, and basal holozoans.

Expanded validation of glass sponge species confirmed these strong matches are not present in other glass sponges. Further, testing showed that even closely related Lyssacinosida species either did not share the matches at all or only presented weak alignments.

These genetic signatures, combined with the existing understanding of *Oopsacas minuta*'s unusual physiology and genome, seem to indicate that *Oopsacas minuta* represents an amoebozoan lineage that achieved permanent multicellular organization independently of metazoan developmental pathways, exhibiting morphological convergence toward sponge-like architecture while retaining fundamental amoebozoan genetic identity.

Keywords

Oopsacas minuta, molecular phylogenetics, BLAST, Amoebozoa, Nuclearia, Fonticula, Hexactinellida, Lyssacinosida, multicellular evolution, eukaryotic systematics, amoebozoan ancestry, metazoan evolution, alternative multicellularity, syncytial organization.

Citation

Gjøvik, Ashley M. "*Oopsacas minuta*, an Amoebozoan Animal: Genetic Evidence Supports a Case of Multicellular Evolution from Amoebozoan Ancestry," *The Journal of Decolonized Ecology and Evolution* 1, no. 1 (July 6 2025). DOI 10.5281/zenodo.15815730

Supplementary Data

Journal: https://osf.io/ur79d/ DOI 10.17605/OSF.IO/UR79D

Issue One Data: https://osf.io/69whf/ DOI 10.17605/OSF.IO/69WHF

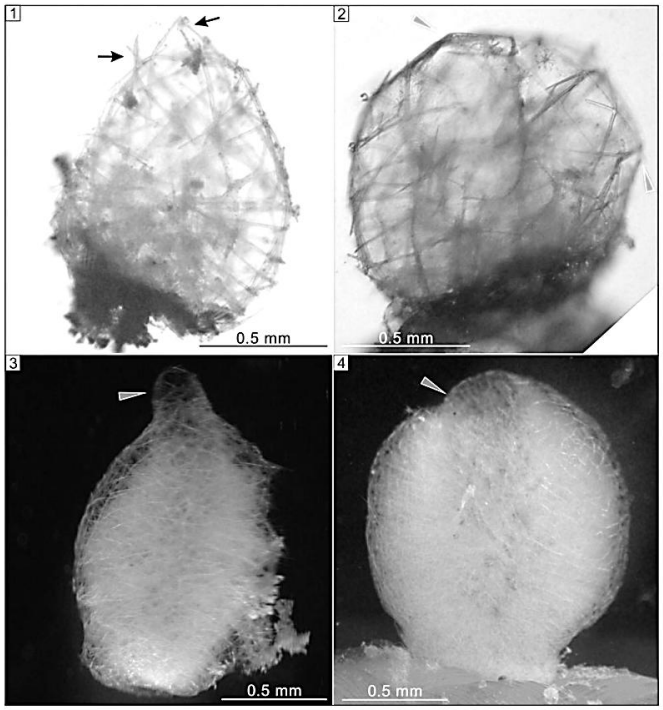
Table of Contents

Introduction

In this study, bidirectional BLAST searches demonstrate that *Oopsacas minuta* exhibits 80-85% sequence identity with nucleariid amoebozoans across multiple conserved markers, including 18S rRNA, 28S rRNA, and mitochondrial genes. These phylogenetic affinities are consistent across forward and reverse search directions, with particularly strong similarity to *Nuclearia thermophila* and *Fonticula alba*. Notably, mitochondrial genome analysis shows 81.1% identity with *N. thermophila* over 1,985 base pairs.

These molecular signatures suggest that *Oopsacas minuta* may represent an amoebozoan lineage that independently achieved multicellular organization. The data indicate possible convergent evolution toward sponge-like architecture through different developmental pathways, warranting reconsideration of the organism's evolutionary origins.

Background: The Anomalous Nature of Oopsacas minuta

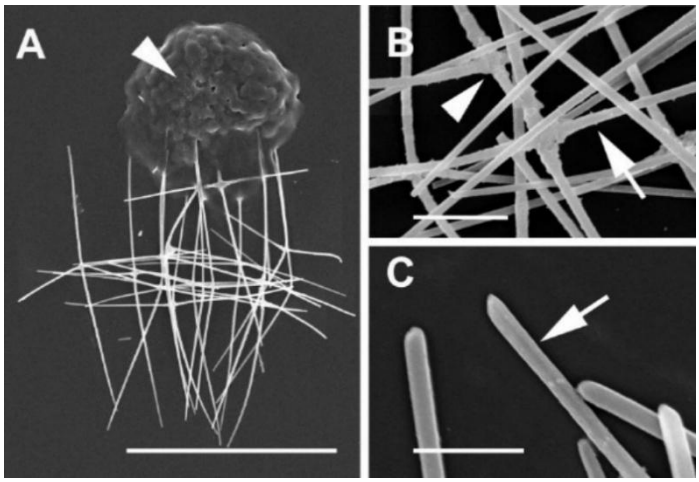


Oopsacas minuta in early life stages (Tang, et al, 2020; Leys, 2007).

Taxonomic Position and Morphological Characteristics

Oopsacas minuta is currently classified within Kingdom Animalia, Phylum Porifera, Class Hexactinellida, Order Lyssacinosida, and Family Leucopsacidae, according to prevailing taxonomic frameworks (Dohrmann et al., 2012). Molecular phylogenetic analyses place Hexactinellida as sister to Demospongiae, with glass sponges exhibiting an ancient divergence from other sponge lineages. Within Hexactinellida, *Oopsacas minuta* is grouped with other lyssacinosidan species characterized by unconnected parenchymal spicules, distinguishing them from hexactinellids with fused skeletal frameworks.

Oopsacas minuta displays morphological characteristics that distinguish it from other sponges. The species exhibits a small cylindrical body measuring 0.5-3.5 cm in length with an elongated, vase-like form showing clear basal-apical polarity (Boury-Esnault & Vacelet, 1994). Unlike typical sponges, *Oopsacas minuta* lacks obvious oscules, with water flow occurring through the sponge wall rather than discrete openings.



Oopsacas minuta. "spicules" and "anterior lipid mass." (Leys, 2003).

Spicular Architecture and Formation

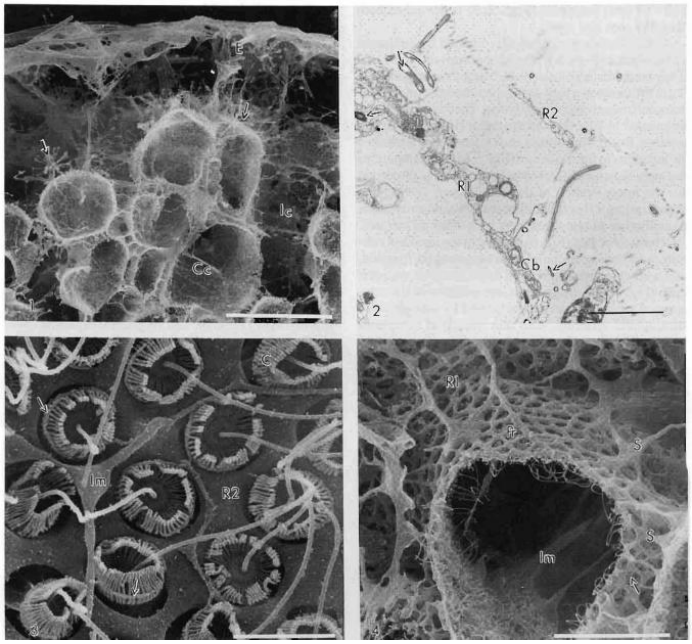
The most striking morphological divergence in *Oopsacas minuta* lies in its spicule formation, which deviates fundamentally from that of typical hexactinellids. In conventional hexactinellids, spicules form within silica deposition vesicles around hexagonal axial filaments containing the protein hexaxilin (López-Escardó et al., 2023). These spicules typically exhibit hexactinic, triaxonic, or cubic symmetry, or derive from such geometries via reduction or branching of primary rays (Lukowiak et al., 2022).

By contrast, *Oopsacas minuta* produces larval spicules organized around rectangular axial filaments. This leads to the formation of four-rayed structures ("stauractins") rather than the characteristic six-rayed hexactines. These stauractins range from 27–45 µm across the transverse rays and 40–80 µm along the longitudinal rays, marking them as significantly smaller and structurally distinct from the megascleres typical of other hexactinellids (Leys, 2003).

Physiological and Developmental Divergence

The physiological and developmental features of *Oopsacas minuta* further distinguish it from canonical hexactinellids and metazoans at large. Unlike most hexactinellids, which inhabit deep-sea environments at depths exceeding 450–900 meters, *Oopsacas minuta* has been found in shallow submarine caves at depths above 200 meters (Leys et al., 2007).

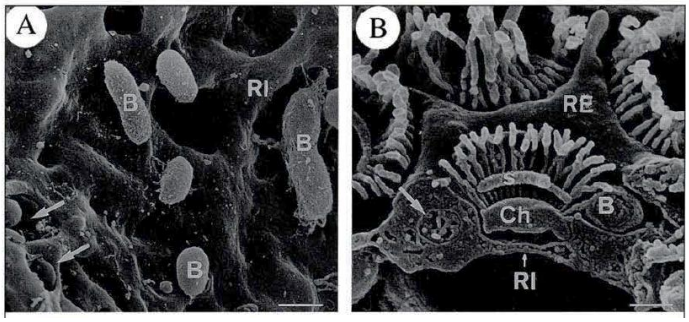
These habitats provide cold, aphotic, and thermally stable conditions ranging from 13 to 14.5 °C (parameters typically associated with deep-sea species). (Vacelet et al., 1994; Bakran-Petricioli et al., 2007). Their discovery in anchihaline cave systems along the French and Croatian coasts supports the hypothesis that such cave environments may act as shallow water "mesocosms" that trap cold, dense water masses and mimic bathyal conditions year-round (Harmelin, 1997).



Oopsacas minuta "Transverse cryofracture through the ectosome and choanosome of a desilicified specimen." (Boury-Esnault, N., & Vacelet, J., 1994).

Oopsacas minuta has unique syncytial tissue architecture. Approximately 75% of the adult body comprises a single multinucleated syncytium, with cytoplasmic continuity maintained through bridges connecting specialized regions. This syncytial reticulum forms both the inner and outer body layers and contains discrete functional zones: archaeocytes for nutrient storage, choanoblasts linked to flagellated chambers, and sclerocytes responsible for spicule formation (Leys et al., 2006).

Embryogenesis in *Oopsacas minuta* proceeds through spiral cleavage and gastrulation via primary delamination. (Boury-Esnault et al., 1999). Remarkably, these reproductive capabilities arise in *Oopsacas minuta* in the complete absence of canonical developmental machinery such as the Wnt signaling pathway and core metazoan transcription factors.



Oopsacas minuta (Perez, T., 1996).

Genomic Simplification

The complete genome of *Oopsacas minuta* reveals a unique case of streamlining, challenging long-held assumptions about the genomic complexity required for animal-grade multicellularity. The mitochondrial genome spans 19 kb with a GC content of 31%. It contains 14 protein-coding genes, three ATP synthase subunits, 22 tRNAs, and two rRNAs. However, *Oopsacas minuta*'s genome notably lacks the *trnaW* gene. (Jourda et al., 2015).

With an estimated genome size of 61 Mb and 16,413 predicted genes, *Oopsacas minuta* possesses one of the smallest non-parasitic metazoan genomes reported. (Santini, S. et al, 2023).

The decreased size is primarily related to the omission of encoding proteins related to enzymatic activities, biomolecule binding properties, and metabolic processes. There was an absence of the canonical Wnt signaling pathway including no *wnt* ligands, *dishevelled*, or *wntless* genes. These are elements thought to be essential even in basal metazoans. (Santini, S. et al, 2023).

The streamlined genome architecture of *Oopsacas minuta* reflects the retention and refinement of a distinct ancestral genomic strategy that supports multicellular organization without reliance on the canonical genetic toolkits of Metazoans.

Critical Absence of Silicification Machinery

The silicification machinery that defines siliceous sponges, particularly within the Hexactinellida, is also missing within *Oopsacas minuta*'s genes (Santini et al., 2023). *Oopsacas minuta* lacks all core genes involved in silica biomineralization, including *silicatein*, *silintaphin*, *galectin*, and *hexaxilin*. In their place, the genome encodes 13 *cathepsin L* genes, a single *glassin* gene, one *ferritin* gene, and

one *chitin synthase* gene.

The absence of *silicatein* is especially significant, as this enzyme catalyzes silica polymerization and forms the axial filaments of spicules (Riesgo et al., 2015). Also missing are *silintaphin* proteins, which interact with *silicatein* to mediate vesicle-bound silica transport and deposition, and *galectins*, which serve as carbohydrate-binding mediators during silica assembly. Further, *Oopsacas minuta* entirely lacks *hexaxilin* and *perisilin*. These are the proteins responsible for establishing spicule symmetry and peripheral silica deposition in glass sponges (López-Escardó et al., 2023; Shimizu et al., 2015).

Methods

This study employed recursive deep homology discovery methodology to systematically identify phylogenetic relationships through long-alignment, recursive, and iterative BLAST searches of *Oopsacas minuta* genomic sequences against NCBI databases. This approach progressively expands search parameters to detect deep evolutionary relationships often missed by conventional phylogenetic methods. (Gjøvik, June 10 2025)

A curated dataset of DNA gene sequences was assembled to evaluate Hexactinellida's relationship in the evolutionary landscape of early complex and multicellular life. Sequences were derived from high-throughput similarity search outputs, with alignment metrics including alignment length (bp), bit score, percent identity, and E-value recorded for each hit. Out of these Hexactinellida results, two strong and curious matches that emerged were *Nuclearia* and *Fonticula*, both strongly matching only *Oopsacas minuta* and this investigation was to validate and understand those matches.

To investigate these genetic relationships of *Oopsacas minuta*, representative nucleotide sequences were obtained from NCBI GenBank and the European Nucleotide Archive. The analysis compiled separate multi-FASTA reference files for each taxonomic group, with reference sequence selection representing well-characterized taxa within each group of interest. *Oopsacas minuta* genomic sequences analyzed included 18S ribosomal RNA (AF207844.1), 28S ribosomal RNA (FR848914.1), and mitochondrial genome (NC_027419.1).

This analysis performed pairwise nucleotide comparisons to assess potential homology between sequences. Two search strategies were employed: standard BLASTn with high-stringency parameters (-evalue 1e-10) and discontiguous MegaBLAST optimized for cross-species comparisons (-task dc-megablast, -evalue 1e-5). All searches generated output in tabular format with comprehensive alignment data including query and subject IDs, alignment length, percent identity, bit score, e-value, mismatch count, coverage metrics, and gap openings.

To prevent false-positive self-alignments, output underwent post hoc filtering using logical conditions excluding self-hits. The resulting alignments were evaluated for biological significance. While BLASTn excels at detecting local sequence similarity, tree-based methods failed to resolve topologies across the dataset due to sequence length constraints, cross-kingdom match profiles spanning protists to holozoans, and missing intermediates from extinct or poorly sequenced lineages.

Results

Strong Amoebozoan Phylogenetic Affinities

Comprehensive bidirectional BLAST analysis revealed consistently strong sequence similarities between *Oopsacas minuta* and nucleariid amoebozoans across multiple molecular markers. These relationships demonstrate remarkable consistency in both forward and reverse search directions, providing robust evidence against contamination or chance similarity while establishing clear phylogenetic affinity with this basal opisthokont clade.

The 18S rRNA analysis between *Oopsacas* and *Nuclearia* species yielded consistently striking results across all tested species. In the forward direction (*Oopsacas* → *Nuclearia*), *Oopsacas minuta* demonstrated 80.6% identity with *N. simplex* over 2,034bp with E=0 (AF207844.1 vs KY454455.1), representing an exceptionally strong match for cross-taxonomic comparison. Similarly compelling was the 81.0% identity with *N. thermophila* over 2,013bp with E=0 (AF207844.1 vs MK547178.1), and the remarkable 84.1-84.4% identity with *N. pattersoni* over 1,140bp with E=0 (AF207844.1 vs LN875110.1).

The reverse direction searches provided validation of these relationships, confirming that the affinities are genuine. *N. thermophila* showed 81.3% identity with *Oopsacas minuta* over 2,020bp with E=0 (MK547178.1 vs JAKMXF010000046.1), while *N. simplex* demonstrated 80.8% identity over 2,035bp with E=0 (KY454455.1 vs JAKMXF010000046.1). *N. pattersoni* similarly showed 80.5% identity over 2,034bp with E=0 (AY364635.1 vs JAKMXF010000330.1). This bidirectional consistency across multiple species and sequence accessions argues strongly against contamination, laboratory error, or chance similarity.

Mitochondrial Genome Conservation

Even more compelling evidence emerges from mitochondrial genome analysis, where the evolutionary conservation patterns provide particularly strong phylogenetic signals. *Nuclearia thermophila* and *Oopsacas minuta* mtDNA showed 81.1% identity over 1,985bp with E=0 (LN875109.1 vs JAKMXF010000330.1), representing an impressive level of conservation for organisms supposedly separated by the vast phylogenetic distance between Amoebozoa and Metazoa.

This level of mitochondrial conservation is phylogenetically significant because mitochondrial DNA evolves slowly and exhibits low tolerance for radical sequence changes compared to nuclear genes. The extensive conservation observed here suggests much more recent common ancestry than would be expected if *Oopsacas minuta* were truly a metazoan sponge. Mitochondrial genes are also less prone to horizontal gene transfer and contamination artifacts, making these matches particularly reliable indicators of genuine phylogenetic relationship rather than convergent sequence evolution or technical artifacts.

Fonticula alba: The Sister Amoebozoan Connection

The relationships extend beyond *Nuclearia* to include *Fonticula alba*, another key amoebozoan lineage that branches sister to *Nuclearia* (Brown et al., 2009). *Fonticula alba* mtDNA showed 69.1% identity over 1,241bp with E-value of 5.85E-134 (JAKMXF010000221.1 vs XM_009498215.1).

Remarkably, the *Fonticula* pre-mRNA-processing-splicing factor 8 (PRPF8) showed 65.0% identity over 5,177bp with a bit score of 1,069 and E=0 (JAKMXF010000210.1 vs XM_009499900.1). This ~5.2 kb sequence alignment between *Oopsacas minuta* and *Fonticula alba* represents a high-confidence match of exceptional length that highlights deep evolutionary conservation of spliceosomal machinery. (PRPF8 is a central catalytic and structural component of the major spliceosome, involved in the precise excision of introns from pre-mRNA).

Further, genomic scaffolds demonstrated 64.8% identity over 3,666bp with bit score of 730 and E=0 (JAKMXF010000210.1 vs NW_009242990.1). In addition, heat shock protein exhibited 65.5% identity over 1,576bp with bit score of 352 and E-value of 5.54×10^{-93} (JAKMXF010000320.1 vs XM_009497412.1). The breadth of these matches across multiple gene categories indicates systematic genetic similarity rather than isolated conservation events.

Comprehensive Protein-Coding Gene Analysis

The *Fonticula alba* protein-coding gene relationships reveal extensive conservation in *Oopsacas minuta* across fundamental cellular machinery. Actin sequences showed conservation at 1,204bp length with 64.9-83% identity across multiple alignments, indicating preservation of this cytoskeletal component. Tubulin demonstrated 74.6% identity over 1,265bp, again reflecting conservation of essential cellular architecture. ATP synthase showed 69.1% identity over 1,241bp, indicating preserved bioenergetic machinery, while cell division proteins exhibited 69.1% identity over 1,401bp, suggesting conserved cell cycle regulation mechanisms.

The pre-mRNA processing factors represent perhaps the most significant finding, with 65% identity over 5,177bp representing the longest high-confidence alignment in the dataset. This level of conservation in spliceosomal machinery indicates that *Oopsacas minuta* shares fundamental gene expression mechanisms with amoebozoan lineages.

Broader Eukaryotic Relationships

Beyond the nucleariid affinities, *Oopsacas minuta* shares strong relationships with basal eukaryotes across multiple lineages. The longest alignments and highest bit scores represent rRNA matches with Filozoa including choanoflagellates and Filasterea.

Notable matches include *Salpingoeca huasca* (1,868bp, 71.9% identity, 959 bit score, E=0), *Tunicaraptor unikontum* (1,120bp, 85% identity, 1,247 bit score, E=0), and *Monosiga brevicollis* (1,118bp, 85% identity, 1,237 bit score, E=0).

There were also substantial mtDNA relationships with multiple Excavata and Filasterea species. These include strong matches with *Ministeria vibrans*, *Malawimonas*, *Imasa heleensis*, Aphelidiaceae, *Histiona aroides*, *Jakoba bahamiensis*, and *Reclinomonas americana*.

The strongest mitochondrial alignments included *Malawimonas* sp. (1,438bp, 69.7% identity, 584 Bit Score, and E-value $1.51E^{-163}$) and *Imasa heleensis* (1,317bp, 69% identity, 498 Bit Score, and E-value $1.72E^{-137}$).

Validation Through Expanded Database Analysis

The validation of these findings required comprehensive verification through multiple database searches and source authentication. All top matched sequences were verified from source listings on NIH databases, with metadata carefully reviewed to ensure data integrity and exclude potential contamination artifacts. The source

authentication reveals a pattern of independent sequencing efforts across multiple laboratories and geographic locations, strengthening confidence in the genuine nature of these relationships.

Source Verification and Data Integrity

The *Oopsacas minuta* sequences derive from multiple independent sources, providing robust validation against laboratory contamination. Primary sequences include PRJNA761294 with whole genome shotgun sequencing conducted at the French National Centre for Scientific Research based on samples from the La Ciotat 3PP cave (Santini et al., 2023), and AF207844.1 sequencing of 18S rRNA at the Centre Oceanologie de Marseille in France (Borchiellini et al., 2001).

The *Nuclearia* sequences represent independent isolations from diverse sources: *N. thermophila* MK547178.1 from small subunit rRNA and ITS1 sequencing at Microbiennes, Université Paris Sud-Saclay (Galindo et al., 2019), *N. simplex* KY454455.1 from mass culture of *Scendesmus* at the Institute of Hydrobiology, Chinese Academy of Sciences (2017), and *N. pattersoni* AY364635.1 from 18S rRNA sequencing at the Institute of Parasitology in the Czech Republic (Dyková et al., 2003).

The *Fonticula alba* sequences derive from comprehensive genomic sequencing of specimens collected from dog dung in Grainfield, Kansas, processed at the Broad Institute Genomics Platform of MIT and Harvard (2013).

Comprehensive Database Coverage: Recursive BLAST Analysis

To understand the full evolutionary context of these relationships, the *Oopsacas minuta* 18S rRNA sequence (AF207844.1) was subjected to comprehensive recursive BLAST analysis against multiple specialized databases. Searches against NCBI core_nt and nt_euk databases yielded matches that were limited to Hexactinellid glass sponges.

The nt_others database searches revealed matches that expand the phylogenetic picture. Strong matches included Suberitida demosponges *Hymeniacidon* and *Halichondria*, Chlorophyta green algae *Chlorolobion lunulatum* and *Polytoma oligochromatum*, and notably, 18S rRNA sequences from fungi including *Trichophyton tonsurans*. The *Trichophyton sp.* match is interesting as a recent study found another *Trichophyton* species to have close genetic affinities with basal eukaryotes. (Gjøvik, June 22, 2025).

There was also a moderate match in the nt_viruses database with White Spot Syndrome Virus (588 bit score, 81.6% identity, and e-value of $7e^{-163}$).

The *Fonticula alba* mRNA sequence for "pre-mRNA-processing-splicing factor 8" that matched the 5,177bp alignment with *Oopsacas minuta* was also used to search the same NIH databases. The core_nt and nt_euk search results predominantly predicted hypothetical proteins with the exception of strong matches to Discosea amoeba *Acanthamoeba castellanii*, Pavlovaceae haptophyte algae *Rebecca billardiae,* picoplankton *Micromonas*, and chytrid *Polychytrium aggregatum*.

No remarkable results were observed in the nt_prok, nt_viruses, or nt_others results with the exception to repeated but weak matches to the *Nocardia sp.* filamented bacteria (which is known to colonize the oral cavities of *Homo sapiens*).

Relationship with Hexactinellid Glass Sponges

The *Oopsacas minuta* mtDNA sequence was run against a custom db containing sequences from representative Hexactinellid species. For context, the self-match was 19,042bp in length with a Bit Score of 35,165.

In comparison, the strongest non-self matches did not exceed 9,000bp or a 6,000 Bit Score. The top matches included the mtDNA (not RNA) of Euplectellidae and Rossellidae groups within Lyssacinosida. (Note: the two other species in the Leucopsacidae group currently appear to be lacking sufficiently detailed genomic data to properly include in the results, with no public sequences exceeding 2,000bp).

The longest-alignment match was a mtDNA alignment with the Euplectellidae species *Vazella pourtalesi* (8,963bp, 74% identity, 5,374 Bit Score, E=0). The highest Bit Score match was to Euplectellidae species *Bathydorus laniger* (6,693bp, 80.8%, 5.986 Bit Score, E=0). The next strongest matches were Rossellidae species *Caulophacus iocasicus*, *Sympagella nux*, and *Bolosoma cyanae* (5,503bp-8,963bp, 79.7-80.5% identity, 3,743-5,855 Bit Scores, and E=0). When aligning these same mtDNA sequences to each other on NIH BLAST web, the Bit Scores and identity %s were within this range, implying their matches to *Oopsacas minuta* align with their matches across their clade.

The *Oopsacas minuta* NCBI core_nt and nt_euk results discussed previously included strong matches, Leucopsacidae (*Leocopsacus*) and Rossellidae (*Acanthascus, Asconema, Nodastrella,* and *Bathydorus*).

Comparative Protist Ancestry Testing Across Glass Sponge Lineages

To determine whether *Oopsacas minuta*'s amoebozoan genetic signatures represented a unique evolutionary

history or reflected broader patterns across glass sponges, comparative BLAST analysis against nucleariid and cercozoan databases was conducted. The results revealed that *Oopsacas minuta* uniquely exhibited strong matches to both lineages. In both tests, there were not confident matches with other hexactinellids. The strongest matches were Lyssacinosida , and specifically *Oopsacas minuta*.

The strongest Cercozoan matches were between *Oopsacas minuta* and unicellular, biflagellated protist *Orciraptor agilis*, and flagellated chlorarachniophyte algae *Bigelowiella natans* and *Lotharella globosa*. (5,560bp+ 69.5+% identity, 2,261+ Bit Score, E=0).

Other lyssacinosidan glass sponges (*Caulophacus, Walteria, Flavovirens*) showed weaker cercozoan matches primarily to *Paulinella microspora*, with significantly lower bit scores and confidence levels. The *Oopsacas-Orciraptor* connection emerged as uniquely robust among glass sponges.

Prokaryotic Database Matches: Ancient Evolutionary Signals

The nt_prok database searches yielded an unexpected array of prokaryotic matches that may reflect ancient evolutionary relationships or horizontal gene transfer events. Strong matches included crenoarchaeotes *Hyphertherthermus*, enterobacteria *Klebsiella pneumoniae* and *Edwardsiella*, cyanobacteria *Microcystis*, firmicutes *Staphylococcus aureus* and *Clostridium* sp., γ-proteobacteria *Azotobacter vinelandii*, *Halomonas*, and *Vibrio*, and β-proteobacteria *Hydrogenophaga*.

A variety of Hexactinellida species (including *Oopsacas minuta* sequences) were also run against a custom db of fungi sequences with a focus on basal and novel species. The strongest matches primarily consisted of *Oopsacas minuta* sequences and top matches included predatory fungi *Arthrobotrys conoides* which is known for tool use and building traps (1,158bp, 82% identity, 1,150 Bit Score, E=0), Kickxellomycotina parasitic fungi *Dimargaris xerosporica* (1,887bp, 66.7% identity, 526 Bit Score, and E-Value of $2.61E^{-145}$), and chytrid *Spizellomyces punctatus* (1,709bp, 68.9% identity, 656 Bit Score, E=0).

The only other Hexactinellida sponge in the top matches was Rossellidae sponge *Acanthascus dawsoni* who presented much weaker matches, and the top matched fungi were *Geoglossum simile* ("earth tongues") and *Radiomyces spectabilis*.

These prokaryotic and fungi relationships, while requiring careful interpretation, may further reflect the ancient evolutionary heritage of *Oopsacas minuta* and its position at a crucial node in eukaryotic evolution.

Further, the presence of *Arthrobotrys conoides* in these results raises even more uncertainty about the proper placement of the *Arthrobotrys* species. While microscopic and characterized as fungi, it does appear to think and behave like an animal, similar to slime mold.

Custom Amoeboid Database: Reinforcement of Amoebozoan Affinities

When *Oopsacas minuta* sequences were analyzed against a comprehensive custom database of amoeba and amoeboid species, the results provided overwhelming reinforcement of the nucleariid relationships. The longest alignment match was to 18S rRNA *Nuclearia thermophila* (1,990bp, 80.7% identity, E=0), confirming this as the strongest single relationship across all database searches. The second longest alignment was to *Gromia oviformis* (1,181bp, 77.8% identity, E=0), another amoeboid organism that extends the pattern of amoebozoan affinities.

The highest bit score match was again to *Nuclearia thermophila* (bit score 1,799), establishing this relationship as the strongest detectable through BLAST methodology. Significantly, the second and third highest Bit Score matches were to other amoeboid organisms: the silica-scale bearing filose amoeba *Pompholyxophrys punicea* (1,105bp, 84.9% identity, bit score 1,232, E=0) and gymnamoebae *Vermistella antarctica* (1,147bp, 82.3% identity, bit score 1,100, E=0).

When *Oopsacas minuta* sequences were analyzed against a Cercozoa-specific custom database, the results showed strong, long-alignment matches (6,000bp+, 68.3-69.8% identity, 2,300+ Bit Score, E=0) between *Oopsacas minuta* WGS DNA and the TSA RNA for the flagellated chlorarachniophyte alga *Bigelowiella natans* and the gliding, algivorous flagellate *Orciraptor agilis*.

Protein-Level Validation and Functional Analysis

REBLAST analysis of apparent spicule-specific *Oopsacas minuta* proteins provided weak matches. The *Oopsacas minuta* glassin protein (KAI6653225.1) matched *Vazella pourtalesii* with a bit score of 398, 52.7% identity, and e-value of $5e^{-132}$, representing a relatively weak match that likely reflects generic protein domain similarity rather than functional homology.

An iron-sulfur cluster assembly protein (KAI6645809.1) showed moderate matches to the jewel wasp *Nasonia vitripennis* (144 bit score, 46.9% identity, $4e^{-40}$ e-value) and basal walking stick *Timema tahoe* (144bp, 53.5% identity, $1e^{-39}$ e-value).

The *Oopsacas minuta* chitin proteins (KAI6645780.1, KAI6646189.1) showed only low-quality matches (<35%

identity) when subjected to blastp analysis. Matches mostly included sponges, stony coral, and bacteria.

Systematic Analysis Conclusion: Convergent Lines of Evidence

There are 10 SRA runs for *Oopsacas minuta* available on NIH databases using both PacBio and Illumina sequencing technologies, providing multiple independent sources for validation. Analysis of short fragments consistently reported ≥95% of reads matching unidentified "dark matter" with top matches to various bacteria. (e.g., SRR23032957, SRR23032961).

Illumina runs on 3-11 kb fragments reported 61.2-64.4% "dark matter," with top identified matches to primates including *Homo sapiens,* crinoid *Anneissia japonica*, coccolithophore *Paramecium tetraurelia*, apicomplexan protists *Plasmodium gaboni* and *Babesia ovata,* cilate *Paramecium tetraurelia,* Stramenopiles diatom *Thalassiosira pseudonana*, fungi Mucoromycota and *Rhizophagus irregularis,* and *Klebsiella sp.* Enterobacteriaceae. The only Porifera match was weak and to demosponge *Amphimedon queenslandica*. (SRA: SRR23032958, SRR23032952, SRR23032960).

This pattern of extensive "dark matter" in sequence databases, combined with the unexpected taxonomic breadth of matches, supports the interpretation that *Oopsacas minuta* represents a highly divergent lineage that does not fit comfortably within existing taxonomic frameworks. The presence of significant unidentifiable genetic material suggests that this organism retains ancient genetic signatures that have been lost in most other eukaryotic lineages, consistent with its proposed status as a multicellular prototype. (Gjøvik, June 23, 2025).

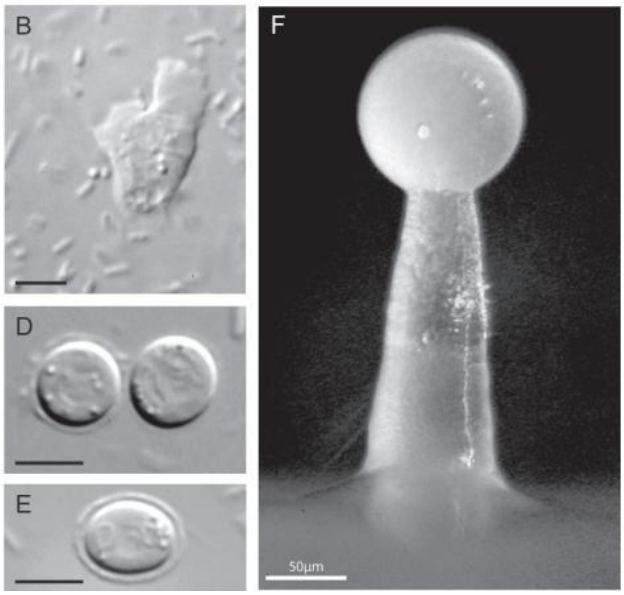
The convergent lines of evidence from multiple database searches, independent sequencing efforts, diverse geographic sources, and varied analytical approaches all point to the same conclusion: *Oopsacas minuta* shares fundamental genetic architecture with amoebozoan lineages.

Discussion

A Novel Amoebozoan Animal

The comprehensive molecular evidence presented in this study compels a radical reinterpretation of the evolutionary identity of *Oopsacas minuta*. Rather than constituting a derived member of the hexactinellid sponges, the data indicate that *Oopsacas minuta* represents a distinct amoebozoan lineage that independently achieved multicellular organization and converged morphologically with sponge body plans via entirely different developmental and genetic mechanisms.

Understanding this reinterpretation requires situating *Oopsacas minuta* within its amoeboid relatives. Nucleariids are recognized as the earliest-diverging lineage of Holomycota, forming a sister group to fungi in modern phylogenomic analyses (Galindo et al., 2019; Gabaldón et al., 2022). *Fonticula alba*, a cellular slime mold, branches sister to *Nuclearia* (Brown et al., 2009). Both lineages display convergent traits that prefigure sponge-like organization, offering a plausible evolutionary bridge to the architecture observed in *Oopsacas minuta*.

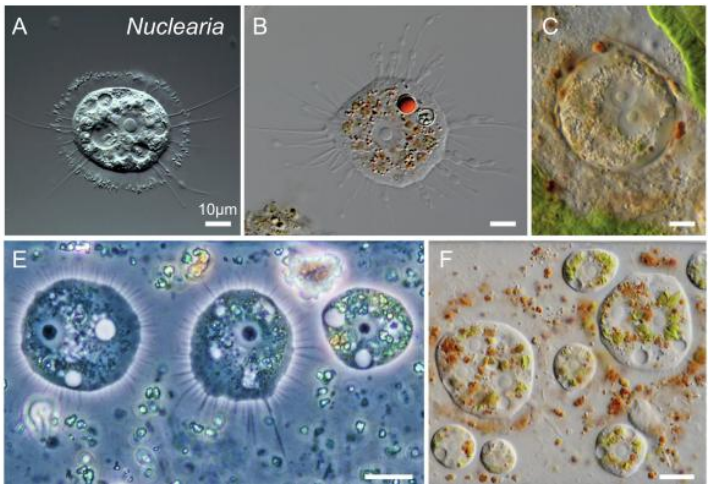


Fonticula alba, (Matthew W. Brown and Alexander K. Tice via Gabaldon, T. et al., 2022)

Fonticula exhibits aggregative multicellularity, forming coordinated fruiting bodies through collective cell movement and differentiation. It feeds on bacteria, secretes extracellular matrix for structural integrity, and demonstrates leader–follower dynamics during collective resource acquisition (Toret et al., 2022).

Nuclearia species, engage in phagocytic filter feeding, often in association with bacterial symbionts, and have been documented colonizing metazoan tissues (Dirren et al., 2017; Galindo et al., 2019). These ecological behaviors mirror those seen in sponges, particularly in their feeding strategies and host associations, further underscoring their relevance as comparative models for *Oopsacas minuta*.

Perhaps the most decisive evidence comes from the mitochondrial genome. The 81.1% sequence identity between *Oopsacas minuta* and *Nuclearia thermophila* across 1,985 base pairs is extraordinarily high, particularly given the slow mutation rate and conservative evolution of mitochondrial DNA.



Nuclearia (Gabaldon, T. et al., 2022)

Amoebozoan Multicellularity and the Multiple Prototype Hypothesis

The identification of *Oopsacas minuta* as a multicellular organism derived from an amoebozoan lineage compels a radical rethinking of how animal-grade complexity can evolve. This clade appears to have originated not in the metazoan lineage, but within Amoebozoa. Its existence offers direct evidence that complex life can arise independently from entirely different genomic and cellular starting points.

The evolutionary architecture of *Oopsacas minuta* challenges nearly every assumption about what is required to build an animal. Traits often thought to demand canonical metazoan signaling machinery are all present, yet the underlying genetic pathways typically responsible for them do not appear to exist.

Oopsacas minuta lacks the Wnt signaling pathway and it is missing numerous transcription factors considered indispensable for early animal development. Still, it executes complex physiological coordination. This is not a degenerate metazoan but a fully functional, coherent multicellular lifeform that simply took another route.

This brings us to the broader theoretical implication: *Oopsacas minuta* substantiates the Multiple Multicellular Prototype (MMCP) hypothesis, which proposes that functionally complex multicellular organisms emerged independently in multiple eukaryotic lineages. These were not divergent branches from a single multicellular ancestor but was a parallel radiation from distinct unicellular progenitors (Gjøvik, June 9 2025).

Under this framework, the features we associate with animals are not limited to a single evolutionary path but represent recurring solutions to the problem of scaling up life.

The molecular evidence supports this view. *Oopsacas minuta* did not evolve from the same Multicellular Prototype as canonical metazoans. While most animals are assumed to have descended from a holozoan LECA, *Oopsacas minuta* appears to have evolved from an amoebozoan LECA closely related to *Nuclearia* and *Fonticula*.

Nuclear and mitochondrial markers show 80–85% sequence identity, 0 e-values, and long alignments with nucleariid genomes. The structural, ecological, and physiological parallels appear extensive.

Moreover, its genomic affinities reach beyond Amoebozoa. Matches with Excavata, Filasterea, Fungi, and even bacterial sequences point to deep genetic retention from early eukaryotic evolution. These wide-ranging similarities reflect *Oopsacas minuta*'s position as a living fossil of an ancient evolutionary trajectory.

In this light, *Oopsacas minuta* is not an outlier but a representative of a broader principle: the transition to animal-grade multicellularity has occurred more than once, through different developmental toolkits and ancestral conditions. At minimum, we now have evidence for two major paths (holozoan and amoebozoan) each capable of producing organisms that meet the functional definition of "animal."

The MMCP framework reframes the story of animal evolution as a branching field of experiments; some successful, some forgotten, and one, in the case of *Oopsacas minuta*, hiding in plain sight.

Alternative Explanations for Molecular Similarities

While the genomic and biochemical evidence overwhelmingly supports an amoebozoan ancestry for *Oopsacas minuta*, the strength of this conclusion necessitates careful consideration of alternative explanations.

The shared mtDNA alignment between *Oopsacas minuta* and other Lyssacinosida sponges was stronger than the nucleariid matches, however these species did not share strong RNA matches. The cross-Lyssacinosida mtDNA matches were of the same quality across the group generally. Further, *Oopsacas minuta* has a smaller genome than the other species, complicating the comparisons.

The other Lyssacinosida species do not match the same amoeboid species that *Oopsacas minuta* does and any matches are also weaker. This all points towards *Oopsacas minuta* either being the first successful experiment in the group or representing a species that began to evolve from a similar gene pool, but took a different direction than its

relatives.

Horizontal gene transfer (HGT) operates under specific mechanistic constraints that limit its explanatory power for the observed patterns. HGT is well-documented among prokaryotes and occurred extensively during early eukaryotic evolution, particularly involving endosymbiotic bacteria and archaeal partners. However, HGT becomes increasingly constrained in complex eukaryotes due to compartmentalization of genetic material, sexual reproduction cycles that eliminate foreign DNA, and integrated gene regulatory networks.

The matches to eukaryotes do not challenge the theory of Amoebozoan animal evolution. Instead, the eukaryote matches are lower quality then the nucleariid with lower bit scores and lower length. They instead represents *Oopsacas minuta* reflecting the shared gene pool of the multiple Multicellular Prototypes.

The sequence similarities between *Oopsacas minuta* and nucleariid amoebozoans span nuclear ribosomal RNA genes, mitochondrial genomes, and diverse protein-coding sequences. HGT explaining these patterns would require coordinated transfer events across nuclear and mitochondrial compartments is mechanistically implausible given that these operate through different pathways and selective pressures. The 80-85% sequence identities reflect systematic conservation across thousands of base pairs, characteristic of phylogenetic inheritance rather than isolated gene acquisitions typical of HGT events.

Oopsacas minuta sequences were independently generated from geographically distinct populations using multiple sequencing technologies by separate research groups. Corresponding nucleariid and *Fonticula* sequences were similarly isolated and sequenced independently. The breadth of molecular similarities across genes, lineages, and data sources exceeds the local, sporadic patterns typical of contamination.

The 80-85% sequence identity observed across large rRNA and mitochondrial fragments exceeds thresholds expected for convergent evolution. Convergent sequence evolution in highly conserved loci is orders of magnitude less likely than morphological convergence under shared ecological pressures.

The 'gene loss' hypothesis (that basal organisms once possessed complex developmental machinery and subsequently lost it) represents a counterintuitive evolutionary scenario. From a parsimony perspective, it is more likely that these genes evolved later as organisms became more complex, rather than appearing early only to be selectively eliminated. If *Oopsacas minuta* represents an early branching point in this evolutionary trajectory, the absence of metazoan developmental systems would reflect ancestral condition rather than secondary loss.

While ancient eukaryotic features might explain some conserved elements, this hypothesis cannot account for the degree and specificity of matches, the complete absence of silicification machinery, or the presence of amoebozoan-specific scaffold proteins. Long-term conservation of multiple independent loci without functional constraint across billion-year divergences is improbable.

The molecular evidence consistently indicates genuine evolutionary affinity between *Oopsacas minuta* and amoebozoan lineages.

Research Gaps and Taxonomic Bias

Several factors explain why *Oopsacas minuta*'s true evolutionary history has remained undetected for nearly a century. Contemporary molecular systematics focuses primarily on resolving relationships within established taxonomic groups rather than questioning whether those groups are correctly defined.

Further, the type of comprehensive exploratory sequence analysis that revealed these relationships is rarely conducted in current research programs. There is minimal focus on exploratory molecular research in current funding and publication priorities, yet evolutionary developmental biology fundamentally requires such exploration to identify novel relationships and challenge existing frameworks.

To address this gap, the recursive deep homology discovery methodology employed here systematically searches across molecular databases to detect deep evolutionary affinities that narrow approaches miss.

Lyssacinosida as Ancestral Sponge Architecture

Existing research places *Oopsacas minuta* within the family Leucopsacidae of the order Lyssacinosida (Dohrmann et al. 2023). Lyssacinosida are thought to be a "stem" group and the earliest hexactinellids, with fossils identified dated to the Late Ordovician (Botting et al. 2025; Li et al. 2019).

Recently, Rossellidae fossils have been identified from the Hirnantian Anji Biota of Zhejiang, China, including what appeared to be a "missing link" relative between demosponges and hexactinellids. (Botting et al., 2017; Botting et al. 2022; Botting et al. 2025).

The lyssacine skeletal organization is characterized by parenchymal spicules usually being unconnected, unlike in other sponges in the subclass where the spicules form a more or less tightly connected skeleton. Recent phylogenetic analyses reveal that the major division of

Hexasterophora is not between lyssacine and dictyonal taxa, but instead between taxa with and without sceptrules and uncinates, placing lyssacinosidans in the basal position (Dohrmann et al. 2017).

The syncytial tissue organization characteristic of hexactinellids finds its most extreme expression in lyssacinosidans, with the majority of the tissue of the adult consisting of a single giant multinucleated syncytium (Leys et al. 2006). This cellular architecture, combined with the absence of electrical signalling mechanisms known in other sponge classes (Leys et al. 2007), suggests retention of protist-like cellular coordination mechanisms.

The embryological development of *Oopsacas* shows formation of syncytia by fusion of blastomeres (Leys et al. 2006), a process that parallels colonial protist organization more closely than typical metazoan embryogenesis.

The high genetic similarity scores between *Oopsacas minuta* and specific rossellid genera compared to other glass sponges (Santini et al. 2023), suggests these lineages share conserved genetic modules that have been lost or diverged in other hexactinellid lineages. This pattern of conservation across supposed family boundaries indicates shared ancestry that predates the radiation of modern hexactinellid families, consistent with retention of ancestral protist-derived genetic architecture.

These findings collectively support the hypothesis that lyssacinosidan "glass sponges" represent either extremely early metazoan forms that retain protist-like organization or constitute a separate lineage of protist descendants that convergently evolved sponge-like morphologies while maintaining fundamental differences in genomic organization and cellular architecture from metazoans.

In addition, within the lyssacinosidan assemblage, *Oopsacas minuta* represents a particularly striking case of either evolutionary divergence from the putative common ancestor or evidence that some core metazoan processes can and do occur without metazoan genetic toolkits.

Under the first theory, the combination of advanced biology with reduced genomic complexity suggests that *Oopsacas* represents an alternative evolutionary solution to multicellular organization that diverged early from other lyssacinosidans and continued evolving along a novel trajectory.

Under the second theory, advanced processes once thought to solely belong to metazoan can and do occur without core metazoan genes, signaling that some of the complex biology functions that we attribute to "animals" are not actually exclusive to metazoans.

The genome of *Oopsacas minuta* provides compelling evidence for protist-like cellular organization. At only 16,413 genes, it is among the smallest of non-parasitic metazoan genomes reported so far, with the absence of many genes that are typically considered ancestral and essential for metazoan morphogenetic processes, including a functional Wnt pathway and numerous transcription factors (Santini et al. 2023). This genomic architecture is consistent with retention of a protist-like genetic toolkit that preceded the elaboration of complex metazoan developmental machinery.

Both theories indicates that the lyssacinosidan assemblage may encompass multiple independent evolutionary experiments in the transition from protist-like to animal-like organization.

Future Research Directions

The proposed reclassification of *Oopsacas minuta* as a multicellular amoebozoan animal demands rigorous experimental confirmation and expanded research.

Further, while molecular and morphological evidence point to an organic, non-siliceous architecture, definitive chemical validation remains essential. Analytical techniques such as energy-dispersive X-ray spectroscopy, X-ray diffraction, FTIR spectroscopy, and hydrofluoric acid dissolution assays should be prioritized to determine whether its spicules are composed of silica or organic matrix.

If it's found this organism does not even use silica like glass sponges do, it would necessitate its removal from Hexactinellida, likely leading to temporary assignment as *Porifera incertae sedis* or even justification for establishing a new high-level taxonomic group of non-metazoan multicellular organisms. This could also finally provide a proper taxonomic home for slime mold.

Parallel research efforts should include expanded phylogenomic sampling across siliceous and non-siliceous sponges to resolve deep relationships and test for other misclassified lineages, and functional studies of its scaffold-building enzymes and reproductive pathway.

Preservation is now an urgent scientific priority. *Oopsacas minuta*'s apparent confinement to Mediterranean anchialine caves (fragile, low-energy habitats) renders it acutely vulnerable to anthropogenic disturbance, ocean acidification, and regional warming. Culture protocols should be developed to allow for development, study, care, and long-term conservation.

This organism may be the sole surviving representative of a billion-year-old evolutionary pathway, and its continued survival must not be left to chance.

Conclusions

This study presents molecular evidence that challenges the current classification of *Oopsacas minuta*. The organism exhibits consistent and unusually strong sequence homology with the amoebozoans *Nuclearia thermophila* and *Fonticula alba*. These affinities appear across multiple conserved loci, including 18S and 28S rRNA, mitochondrial sequences, and a 5.2 kb region of PRPF8—none of which are expected in a metazoan lineage.

The combination of amoebozoan molecular identity, absence of key metazoan developmental systems, and independently evolved multicellular features supports the hypothesis that *Oopsacas minuta* represents a distinct evolutionary lineage. These traits are not easily explained by sponge ancestry alone and appear to reflect convergence on sponge-like physiology from a different starting point.

Oopsacas minuta and the broader lyssacinosidan assemblage represent a remarkable case of convergent evolution toward animal-level complexity through an entirely non-metazoan evolutionary pathway. Genetic alignments between *Oopsacas* and amoebozoans like *Nuclearia* and *Fonticula alba*, combined with the group's extensive syncytial architecture and unique capabilities, suggest these organisms achieved animal-like functions through amoebozoan cellular machinery (i.e., cytoplasmic network signaling, and multinucleated syncytial organization) rather than canonical metazoan developmental pathways.

The evolutionary trajectory appears to progress from *Oopsacas*-like prototypes using pure amoebozoan genetics to achieve animal functions, through lyssacinosidan transitional forms that acquired metazoan genes while retaining amoebozoan architectures, to later glass sponges that "metazoan-ized" these originally amoebozoan innovations.

This represents independent evolution of multicellular complexity parallel to conventional metazoans, supporting the Multiple Multicellular Prototype hypothesis. Rather than being degenerate animals missing essential genes, glass sponges appear to be successful amoebozoans that invented animal-like ecological strategies before metazoans did, subsequently influencing or converging with metazoan evolution while maintaining their distinctive amoebozoan cellular heritage.

Acknowledgements

The author conceptualized the study, developed the theoretical framework, and conducted all analyses. This research received no external funding and was conducted using personally acquired resources. No new data was created for this article.

The author thanks the open-access genomic resources and databases that made this analysis possible, particularly the *Oopsacas minuta* genome projects and NCBI sequence repositories.

The author acknowledges Claude AI, Molecular Biologist GPT, and Scholar GPT for their assistance with manuscript preparation, data interpretation discussions, and research development over the course of this investigation.

References

Anderson, Oopsacas. (2018). A Half-century of Research on Free-living Amoebae (1965-2017): Review of Biogeographic, Ecological and Physiological Studies. Acta Protozoologica. 57. 1-28.

Bakran-Petricioli, T., Vacelet, J., Zibrowius, H., Petricioli, D., Chevaldonné, P., & Raa, T. (2007). New data on the distribution of the "deep-sea" sponges *Asbestopluma hypogea* and *Oopsacas minuta* in the Mediterranean Sea. *Marine Ecology*, 28(Suppl. 1), 10-23.

Belahbib H, Renard E, Santini S, et al. New genomic data and analyses challenge the traditional vision of animal epithelium evolution. *BMC Genomics*. 2018;19(1):393.

Borchiellini, C., Manuel, M., Alivon, E., Boury-Esnault, N., Vacelet, J., & Le Parco, Y. (2001). Sponge paraphyly and the origin of Metazoa. *Journal of evolutionary biology*, *14*(1), 171–179.

Botting, Joseph & Janussen, Dorte & Dohrmann, Martin & Muir, Lucy & Zhang, Yuandong & Junye, Ma. (2025). Advanced crown-group Rossellidae (Porifera: Hexactinellida) resembling extant taxa from the Hirnantian (Late Ordovician) Anji Biota. Papers in Palaeontology. 11.

Botting, Joseph. "Cyathophycus and the Origin of Demosponges." Lethaia, Taylor & Francis, 2003.

Botting, Joseph P., Yuchen Lu, Lucy A. Muir, Junfeng Guo, Guwei Li, Chao Chang, and Peiyun Cong. "A Probable Hyalonematid Sponge (Hexactinellida: Amphidiscophora) from the Middle Ordovician of the Builth Inlier, Wales." *Palaeoworld* 31, no. 4 (2022): 704-722.

Botting, Joseph & Zhang, Yuandong & Muir, Lucy. (2017). Discovery of missing link between demosponges and hexactinellids confirms palaeontological model of sponge evolution. Scientific Reports. 7.

Boury-Esnault, N., Efremova, S., Bézac, C., & Vacelet, J.

(1999). Reproduction of a hexactinellid sponge: first description of gastrulation by cellular delamination in the Porifera. *Invertebrate Reproduction and Development*, 35(3), 187-201.

Boury-Esnault, N., & Vacelet, J. (1994). Preliminary studies on the organization and development of a hexactinellid sponge from a Mediterranean cave, *Oopsacas minuta*. In R.W.M. van Soest, Th.M.G. van Kempen, & J.-C. Braekman (Eds.), *Sponges in Time and Space* (pp. 407-415). Balkema.

Brown, M. W., Spiegel, F. W., & Silberman, J. D. (2009). Phylogeny of the "forgotten" cellular slime mold, *Fonticula alba*, reveals a key evolutionary branch within Opisthokonta. *Molecular Biology and Evolution*, 26(12), 2699-2709.

Brunet T. Cell contractility in early animal evolution. *Curr Biol*. 2023;33(18):R966-R985.

Clark, H.J. (1868). On the Spongiae Ciliatae as Infusoria Flagellata; or, observations on the structure, animality, and relationship of Leucosolenia botryoides, Bowerbank. *Memoirs of the Boston Natural History Society*. 1(III): 305-340.

Dayel MJ, Alegado RA, Fairclough SR, et al. Cell differentiation and morphogenesis in the colony-forming choanoflagellate Salpingoeca rosetta. *Dev Biol*. 2011;357(1):73-82.

Dirren, S., Pitsch, G., Silva, M. Oopsacas D., & Posch, T. (2017). Grazing of *Nuclearia thermophila* and *Nuclearia delicatula* (Nucleariidae, Opisthokonta) on the toxic cyanobacterium *Planktothrix rubescens*. *European Journal of Protistology*, 60, 87-101.

Dohrmann, Martin & Erpenbeck, Dirk & Larroux, Claire & Maldonado, Manuel & Voigt, Oliver & Borchiellini, Carole & Lavrov, Dennis. (2012). Deep Phylogeny and Evolution of Sponges (Phylum Porifera). *Advances in marine biology*. 61. 1-78.

Dohrmann, Martin, Dorte Janussen, Joachim Reitner, Allen G. Collins, and Gert Wörheide. "Phylogeny and Evolution of Glass Sponges (Porifera, Hexactinellida)." *Systematic Biology* 57, no. 3 (2008): 388-405.

Dohrmann, Martin, Chris Kelley, Michelle Kelly, Andrzej Pisera, John N.A. Hooper, and Henry M. Reiswig. "An integrative systematic framework helps to reconstruct skeletal evolution of glass sponges (Porifera, Hexactinellida)." *Frontiers in Zoology* 14 (2017): 18.

Dohrmann, M., D. Janussen, J. Reitner, A.G. Collins, and G. Wörheide. 2012. Phylogeny and evolution of glass sponges (Porifera, Hexactinellida). *Molecular Phylogenetics and Evolution* 63: 23-34.

Dohrmann, Martin, Henry M. Reiswig, Michelle Kelly, Sadie Mills, Simone Schätzle, Miriam Reverter, Natascha Niesse, Sven Rohde, Peter Schupp, and Gert Wörheide. "Expanded Sampling of New Zealand Glass Sponges (Porifera: Hexactinellida) Provides New Insights into Biodiversity, Chemodiversity, and Phylogeny of the Class." *PeerJ* 11 (2023): e15017.

Dyková I, Veverková M, Fiala I, Macháčková B, Pecková H. Nuclearia pattersoni sp. n. (Filosea), a new species of amphizoic amoeba isolated from gills of roach (Rutilus rutilus), and its rickettsial endosymbiont. *Folia Parasitol (Praha)*. 2003;50(3):161-170.

Fontorbe, G., et al. (2017). The silicon isotopic composition of Ethmodiscus rex laminated diatom mats from the tropical Atlantic: Implications for silica cycling in the equatorial Pacific during the Miocene. *Paleoceanography and Paleoclimatology*, 32, 1-15.

Gabaldón, T., Völcker, E., & Torruella, G. (2022). On the biology, diversity and evolution of nucleariid amoebae (Amorphea, Obazoa, Opisthokonta). *Protist*, 173(4), 125895.

Galindo, L. J., Torruella, G., Moreira, D., Eglit, Y., Simpson, A. G. B., Völcker, E., Clauß, S., & López-García, P. (2019). Combined cultivation and single-cell approaches to the phylogenomics of nucleariid amoebae, close relatives of fungi. *Philosophical Transactions of the Royal Society B*, 374(1786), 20190094.

Gerovasileiou V, Voultsiadou E. Marine caves of the Mediterranean Sea: a sponge biodiversity reservoir within a biodiversity hotspot. *PLoS One*. 2012;7(7):e39873.

Gertraud Burger, Michael W. Gray, Lise Forget, B. Franz Lang, Strikingly Bacteria-Like and Gene-Rich Mitochondrial Genomes throughout Jakobid Protists, *Genome Biology and Evolution*, Volume 5, Issue 2, February 2013, Pages 418–438.

Gjøvik, Ashley M. "The Multiple Multicellular Prototype Framework: Silica-based Biomineralization and Early Eukaryotic Diversification". *The Journal of Decolonized Ecology and Evolution* 1, no. 1 (June 9, 2025). https://doi.org/10.5281/zenodo.15622349.

Gjøvik, Ashley M. "A Recursive Strategy for Deep Homology Discovery in Genetic Sequence Alignment". *The Journal of Decolonized Ecology and Evolution* 1, no. 1 (June 10, 2025). https://doi.org/10.5281/zenodo.15628144

Gjøvik, Ashley, M. "Molecular Characterization of Trichophyton Schoenleinii Reveals Systematic Affinities with Ancient Eukaryotic Lineages." The Journal of Decolonized Ecology and Evolution 1, no. 1 (June 22,

2025). https://doi.org/10.5281/zenodo.15717921.

Gjøvik, Ashley, M. "Compiled Chemistry: Structural Inheritance and the Deep Origins of Biological Function." *The Journal of Decolonized Ecology and Evolution* 1, no. 1 (June 23, 2025). https://doi.org/10.5281/zenodo.15717955

Gong, Lin, Mei Yang, Dorte Janussen, Martin Dohrmann, and Xikun Li. "A new species of Caulophacus (Hexactinellida: Lyssacinosida: Rossellidae) from the western Pacific Ocean, with new insights into the mitochondrial genome characteristics of hexactinellid sponges." *Frontiers in Marine Science* 10 (2023): 979912.

Harmelin JG, Vacelet J (1997) Clues to deep-sea biodiversity in a nearshore cave. Vie Milieu 47: 351–354.

Heiss AA, Kolisko M, Ekelund F, Brown MW, Roger AJ, Simpson AGB. Combined morphological and phylogenomic re-examination of malawimonads, a critical taxon for inferring the evolutionary history of eukaryotes. *R Soc Open Sci*. 2018;5(4):171707.

Hendry, K. R., & Robinson, L. F. (2012). The relationship between silicon isotope fractionation in sponges and silicic acid concentration: Modern and core-top studies of biogenic opal. *Geochimica et Cosmochimica Acta*, 81, 1-12.

Hendry, K. R., Swann, G. E. A., Leng, M. J., et al. (2019). Technical note: Silica stable isotopes and silicification in a carnivorous sponge *Asbestopluma* sp. *Biogeosciences*, 16, 3677-3691.

Krautter, Manfred. "Fossil Hexactinellida: An Overview." In *Systema Porifera*, edited by J.N.A. Hooper and R.W.M. Van Soest, 1211-1223. Boston: Springer, 2002.

Jourda, C., Santini, S., Rocher, C., Le Bivic, A., & Claverie, J. M. (2015). Mitochondrial Genome Sequence of the Glass Sponge Oopsacas minuta. *Genome announcements*, *3*(4), e00823-15.

Leon F, EspinozaEsparza JM, Deng V, Coyle MC, Espinoz a S, Booth DS. 2025. Cell differentiation controls iron assimilation in the choanoflagellate *Salpingoeca rosetta*. mSphere 10:e00917-24.

Leys, S. P. (2003). Comparative study of spiculogenesis in demosponge and hexactinellid larvae. *Microscopy Research and Technique*, 62(4), 300-311.

Leys SP. The significance of syncytial tissues for the position of the hexactinellida in the metazoa. *Integr Comp Biol*. 2003;43(1):19-27.

Leys, S. P., Cheung, E., & Boury-Esnault, N. (2006). Embryogenesis in the glass sponge *Oopsacas minuta*: formation of syncytia by fusion of blastomeres. *Integrative and Comparative Biology*, 46(2), 104-117.

Leys, S. P., Mackie, G. Oopsacas, & Reiswig, H. M. (2007). The biology of glass sponges. *Advances in Marine Biology*, 52, 1-145.

Leys, S. P., Kamarul Zaman, A., & Boury-Esnault, N. (2016). Three-dimensional fate mapping of larval tissues through metamorphosis in the glass sponge *Oopsacas minuta*. *Invertebrate Biology*, 135(3), 259-272.

Li, Luoyang, Dorte Janussen, Renbin Zhan, Jisuo Jin, Mu Liu, Junye Ma, Yuandong Zhang, and Xueping Ma. "Oldest known fossil of Rossellids (Hexactinellida, Porifera) from the Ordovician–Silurian transition of Anhui, South China." *PalZ* 93 (2019): 559-566.

López-Escardó, D., et al. (2023). Silica-associated proteins from hexactinellid sponges support an alternative evolutionary scenario for biomineralization in Porifera. *Nature Communications*, 14, 8145.

Łukowiak M, Van Soest R, Klautau M, Pérez T, Pisera A, Tabachnick K. The terminology of sponge spicules. *J Morphol*. 2022;283(12):1517-1545.

Lundsten, Lonny, Susan von Thun, Linda Kuhnz, Jim Barry, Freya Goetz, George Matsumoto, Henry Reiswig, and Welton Lee. "Time-series analysis of six deep-sea demosponge species from Monterey Canyon reveals species-specific responses to environmental changes." *Deep Sea Research Part I: Oceanographic Research Papers* 96 (2015): 108-117.

Lynn, Robert J. "Seasonal variation of temperature and salinity at 10 meters in the California Current." *California Cooperative Oceanic Fisheries Investigations Reports* 11 (1967): 157-175.

Mackie, G.Oopsacas and C.L. Singla. 1983. Studies on hexactinellid sponges. I. Histology of *Rhabdocalyptus dawsoni* (Lambe, 1873). *Philosophical Transactions of the Royal Society B* 301: 365-400.

Maldonado, M., López-Acosta, M., Beazley, L., Kenchington, E., Koutsouveli, V., & Riesgo, A. (2020). Cooperation between passive and active silicon transporters clarifies the ecophysiology and evolution of biosilicification in sponges. *Science Advances*, 6(28), eaba9322.

Mair, Magdalena & Ruther, Joachim. (2019). Chemical Ecology of the Parasitoid Wasp Genus Nasonia (Hymenoptera, Pteromalidae). *Frontiers in Ecology and Evolution*. 7.

Manuel, M., Le Parco, Y., & Borchiellini, C. (2004). Comparative analysis of Brachyury T-domains, with the characterization of two new sponge sequences, from a hexactinellid and a calcisponge. *Gene*, 340(2), 291-301.

Moran, D.M., Anderson, OopsacasR., Dennett, M.R., Caron, D.A., Gast, R.J. 2007. A Description of Seven Antarctic Marine Gymnamoebae Including a New Subspecies, Two New Species and a New Genus: *Neoparamoeba aestuarina antarctica* n. subsp., *Platyamoeba oblongata* n. sp., *Platyamoeba contorta* n. sp. and *Vermistella antarctica* n. gen. n. sp.*J. Eukaryotic Microbiology* **54**: 169-183

Müller, W. E. G., Eckert, C., Kropf, K., et al. (2007). Formation of giant spicules in the deep-sea hexactinellid *Monorhaphis chuni* (Schulze 1904): electron-microscopic and biochemical studies. *Cell and Tissue Research*, 329(2), 363-378.

Müller, W. E. G., Wang, X., Burghard, Z., et al. (2009). Bio-sintering processes in hexactinellid sponges: fusion of bio-silica in giant basal spicules from *Monorhaphis chuni*. *Journal of Structural Biology*, 168(3), 548-561.

Nielsen C. Six major steps in animal evolution: are we derived sponge larvae?. *Evol Dev*. 2008;10(2):241-257.

Perez, T. (1996). La rétention de particules par une éponge hexactinellide, Oopsacas minuta (Leucopsacasidae): le rôle du réticulum. *CR Acad. Sci. Ser. III Life Sci*, *319*, 385-391.

Pisera, A., Łukowiak, M., Masse, S., et al. (2021). Insights into the structure and morphogenesis of the giant basal spicule of the glass sponge *Monorhaphis chuni*. *Frontiers in Zoology*, 18(1), 58.

Reiswig, Henry M., and Juan Francisco Araya. "A review of the Hexactinellida (Porifera) of Chile, with the first record of Caulophacus Schulze, 1885 (Lyssacinosida: Rossellidae) from the Southeastern Pacific Ocean." *Zootaxa* 3889, no. 3 (2014): 414-428.

Reynolds, Andrew. (2008). Amoebae as Exemplary Cells: The Protean Nature of an Elementary Organism. Journal of the history of biology. 41. 307-37.

Riesgo, A., Maldonado, M., López-Legentil, S., & Giribet, G. (2015). A proposal for the evolution of cathepsin and silicatein in sponges. *Journal of Molecular Evolution*, 80(5), 278-291.

Rodríguez-Ezpeleta N, Brinkmann H, Burger G, et al. Toward resolving the eukaryotic tree: the phylogenetic positions of jakobids and cercozoans. *Curr Biol*. 2007;17(16):1420-1425.

Ros-Rocher N, Pérez-Posada A, Leger MM, Ruiz-Trillo I. The origin of animals: an ancestral reconstruction of the unicellular-to-multicellular transition. *Open Biol*. 2021;11(2):200359.

Rossi, M. Eleonora & et al. (2024). Independent origins of spicules reconcile the evolutionary history of sponges.

Santini, S., Schenkelaars, Q., Jourda, C., et al. (2023). The compact genome of the sponge *Oopsacas minuta* (Hexactinellida) is lacking key metazoan core genes. *BMC Biology*, 21, 139.

Schenkelaars, Quentin & Pratlong, Marine & Kodjabachian, Laurent & Fierro-Constain, Laura & Vacelet, Jean & Le Bivic, André & Renard, Emmanuelle & Borchiellini, Carole. (2017). Animal multicellularity and polarity without Wnt signaling. Scientific Reports. 7. 10.

Shimizu, K., Amano, T., Bari, M. R., et al. (2015). Glassin, a histidine-rich protein from the siliceous skeletal system of the marine sponge *Euplectella*, directs silica polycondensation. *Proceedings of the National Academy of Sciences*, 112(37), 11449-11454.

Shimizu K, Nishi M, Sakate Y, et al. Silica-associated proteins from hexactinellid sponges support an alternative evolutionary scenario for biomineralization in Porifera. *Nat Commun*. 2024;15(1):181.

Simpson AG, Inagaki Y, Roger AJ. Comprehensive multigene phylogenies of excavate protists reveal the evolutionary positions of "primitive" eukaryotes. *Mol Biol Evol*. 2006;23(3):615-625.

Sperling, E. A., Rosales-Lagarde, L., Aubrecht, R., et al. (2019). Statistical analysis of iron geochemical data suggests limited late Proterozoic oxygenation. *Nature*, 523, 451-454.

Tang Q, Hu J, Xie G, et al. A problematic animal fossil from the early Cambrian Hetang Formation, South China. *J Paleontol*. 2019.

Topsent, E. (1927). Diagnoses d'Éponges nouvelles recueillies par le Prince Albert Ier de Monaco. *Bulletin de l'Institut océanographique Monaco*, 502, 1-19.

Topsent, E. (1928). Spongiaires de l'Atlantique et de la Méditerranée provenant des croisières du Prince Albert Ier de Monaco. [Atlantic and Mediterranean sponges from the cruises of Prince Albert I of Monaco]. *Résultats des campagnes scientifiques accomplies par le Prince Albert I. Monaco.* 74:1-376, pls I-XI. page(s): 75-76.

Tong, S. M. (1997). Heterotrophic flagellates and other protists from Southampton Water, U.K. *Ophelia*. 47(2): 71-131

Toret, C. P., Peled, S., Eelkema, R., Kaksonen, M., & Bassereau, P. (2022). The cellular slime mold *Fonticula alba* forms a dynamic, multicellular collective while feeding on bacteria. *Current Biology*, 32(7), 1398-1411.

Uriz, M. J., Turon, X., Becerro, M. A., & Agell, G. (2003). Siliceous spicules and skeleton frameworks in sponges:

origin, diversity, ultrastructural patterns, and biological functions. *Microscopy Research and Technique*, 62(4), 279-299.

Vacelet, J., Boury-Esnault, N., & Harmelin, J.-G. (1994). Hexactinellid cave, a unique deep-sea habitat in the scuba zone. *Deep Sea Research Part I*, 41(7), 965-973.

Wang, X., Schröder, H. C., & Müller, W. E. G. (2009). Giant siliceous spicules from the deep-sea glass sponge *Monorhaphis chuni*. *International Review of Cell and Molecular Biology*, 273, 69-115.

Werren, J. H., & Loehlin, D. W. (2009). The parasitoid wasp Nasonia: an emerging model system with haploid male genetics. *Cold Spring Harbor protocols*, *2009*(10), pdb.emo134.

Wörheide, G., M. Dohrmann, D. Erpenbeck, C. Larroux, M. Maldonado, Oopsacas Voigt, C. Borchiellini, and D. Lavrov. 2012. Deep phylogeny and evolution of sponges (phylum Porifera). *Advances in Marine Biology* 61: 1-78.

Worley, A. C., Raper, K. B., & Kessler, D. (1979). *Fonticula alba*: a new cellular slime mold (Acrasiomycetes). *Mycologia*, 71(4), 746-760.