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Report

Phylogenomics of the Intracellular Parasite *Mikrocytos mackini* Reveals Evidence for a Mitosome in Rhizaria

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Summary

Mikrocytos mackini **is an intracellular protistan parasite of oysters whose position in the phylogenetic tree of eukaryotes has been a mystery for many years [1,2]. *M. mackini* is difficult to isolate, has not been cultured, and has no defining morphological feature. Furthermore, its only phylogenetic marker that has been successfully sequenced to date (the small subunit ribosomal RNA) is highly divergent and has failed to resolve its evolutionary position [2]. *M. mackini* is also one of the few eukaryotes that lacks mitochondria [1], making both its phylogenetic position and comparative analysis of mitochondrial function particularly important. Here, we have obtained transcriptomic data for *M. mackini* from enriched isolates and constructed a 119-gene phylogenomic data set. *M. mackini* proved to be among the fastest-evolving eukaryote lineages known to date, but, nevertheless, our analysis robustly placed it within Rhizaria. Searching the transcriptome for genetic evidence of a mitochondrion-related organelle (MRO) revealed only four mitochondrion-derived genes: *IscS*, *IscU*, *mtHsp70*, and *FdxR*. Interestingly, all four genes are involved in iron-sulfur cluster formation, a biochemical pathway common to other highly reduced "mitosomes" in unrelated MRO-containing lineages [7]. This is the first evidence of MRO in Rhizaria, and it suggests the parallel evolution of mitochondria to mitosomes in this supergroup.**

Results and Discussion

Mikrocytos mackini Is a Derived Rhizarian

Few eukaryotes are of a completely unknown evolutionary origin. Despite ultrastructural and molecular studies, the enigmatic microcell parasite *Mikrocytos mackini*, which is the causative agent of the Denman Island disease in oysters, is one such orphan lineage [1,2]. Recently, molecular detection has shown that diverse *Mikrocytos*-like parasites infect a variety of oysters in different regions of the world, but it did not provide clues of *M. mackini*'s phylogenetic position [3–5]. On the basis of phylogenetic analysis of small subunit (SSU) ribosomal DNA (rDNA), it was tentatively proposed that *M. mackini* was a basal, highly divergent eukaryote without close relatives [2]. This idea was reinforced by the structural simplicity of microcells and the fact that no canonical mitochondrion or related organelle, such as hydrogenosome or mitosome, has been observed [1,6]. However, in the last 10 years, systematic studies of other "amitochondriate" lineages have repeatedly falsified the hypothesis that these lineages ancestrally lack mitochondria. The current view is that all extant eukaryotes possess mitochondria or reduced mitochondrial-related organelles (MROs), which have independently evolved in several lineages (see [7,8] for reviews). To see how *M. mackini* fits into this picture of mitochondrial evolution, we generated a large transcriptome data set by Illumina sequencing and used it to obtain multiple genes in order to investigate its phylogenetic position and assess what type of MRO, if any, is most likely present.

M. mackini cells are not easily separated from its Pacific oyster host, *Crassostrea gigas* [9]. Using an enrichment protocol and the *C. gigas* genome to filter host-derived sequences from the transcriptome (see the Supplemental Information available online), we identified 15,874 contigs of putative *M. mackini* origin. From these, we searched for homologs of 252 protein-coding genes previously used in a phylogenomic study [10], which revealed that *M. mackini* genes are characterized by very high rates of evolution. Consequently, single-gene trees were generally poorly resolved; therefore, we applied two conditions in order to assess orthology of the *M. mackini* sequences: (1) the sequences must be most closely related to the correct orthologs (bootstrap $\geq$ 70%), and (2) they must show no sign of suspicious relationships to any other lineages (such as *C. gigas* or prokaryotes). Overall, this led to the identification of 103 *M. mackini* orthologous sequences, but, because a preliminary analysis showed that *M. mackini* might belong to the eukaryotic supergroup Rhizaria (data not shown), we added an additional 16 genes that had fewer than 60% missing positions for Rhizaria as a whole, leading to a concatenated alignment of 119 genes (Table S1) and 23,162 amino acid (aa) positions.

To determine the relationship of *M. mackini* to other eukaryotes, we first analyzed a broad taxon sampling of 101 operational taxonomic units (OTUs), including representatives of all supergroups and lineages with no strong affiliation, such as *Collodictyon*, *Thecamonas*, or *Picobiliphytes* (Figure 1). This data set also contained notoriously fast-evolving taxa, such as the cryptomonad nucleomorphs, diplomonads, and parabasalians. Phylogenetic analyses using maximum likelihood (ML) with the site-homogeneous LG model and Bayesian method (BI) with the site-heterogeneous CAT model were in general agreement with recent phylogenomic analyses [11–16]. More interestingly, both analyses strongly supported *M. mackini* branching within Rhizaria (1.0 BI posterior probability and 91% ML bootstrap). The extreme rate of evolution of *M. mackini* genes was reflected in its branch length: from a hypothetical root on unikonts (Amoebozoa + opisthokonts), as recently proposed on the basis of mitochondrial and bacterial proteins [17], the distance to *M. mackini* is twice that of the second longest branch in the ML tree (the diplomonad *Spironucleus*). Nevertheless, the position of

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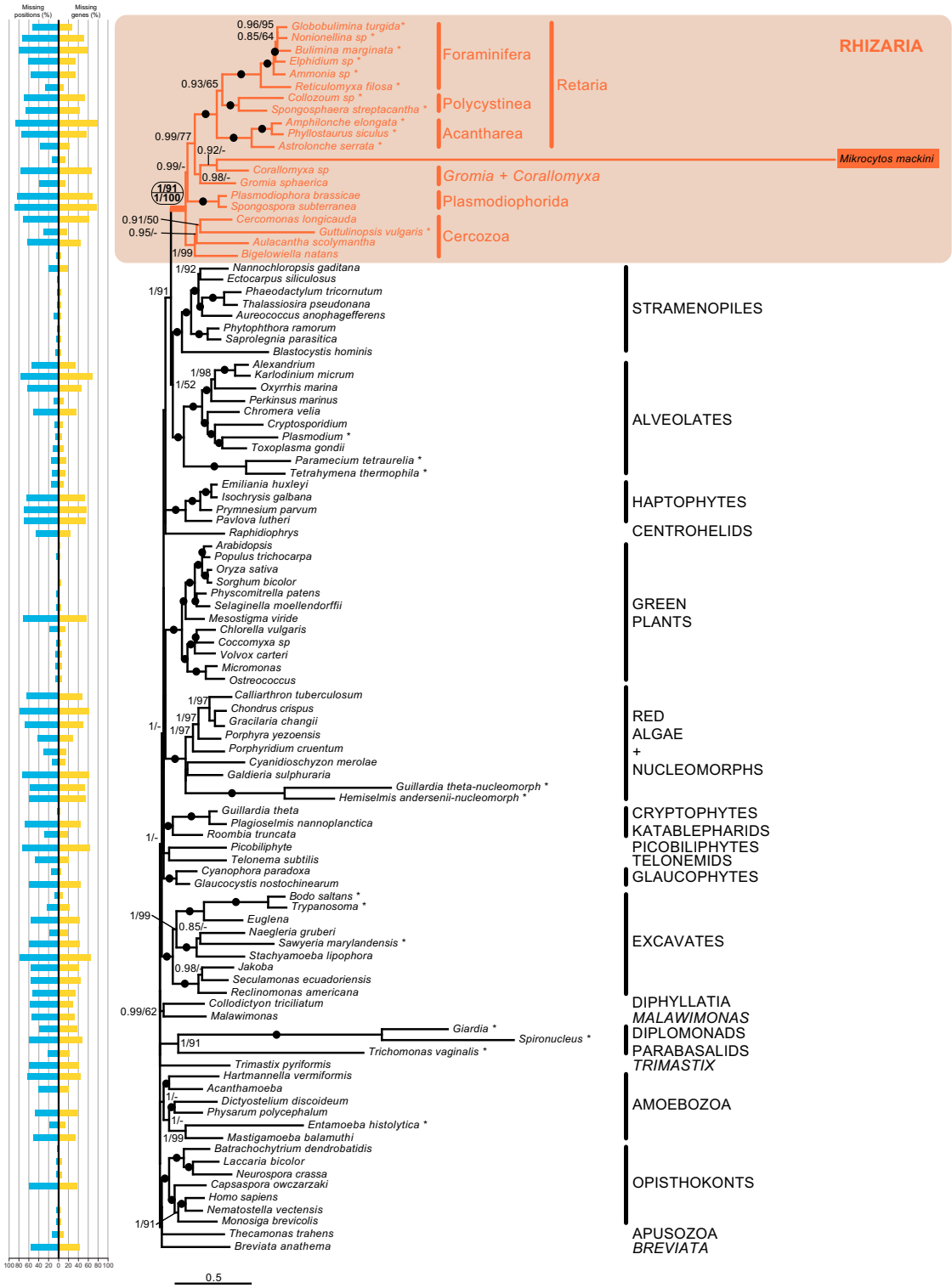

Figure 1. Global Phylogenetic Tree of Eukaryotes

A tree inferred with 119 genes and 101 OTUs under the CAT + Γ4 model, as implemented in PhyloBayes. Two Markov chain Monte Carlo chains were run for 9,000 cycles with a burn-in of 1,000, but convergence was not reached for a few nodes outside of Rhizaria. In comparison to the tree shown here, differences between the chains concerned the relative positions of haptophytes, centrohelids, cryptophytes + katablepharids, picobiliphytes, and telonemids. Values at nodes denote Bayesian posterior probabilities (to the left of the slash) and maximum likelihood bootstrap support (to the right of the slash). Black circles indicate that maximum support was recovered in both methods (i.e., 1/100). The thick internal branch leading to Rhizaria emphasizes strong support for the grouping of *M. mackini* within this supergroup. Support at this node also includes values from the 77 taxa analyses (under the line); i.e., after the removal of the longest branches (denoted with a * after the taxon names). The scale bar represents the estimated number of amino acid substitutions per site. The bars on the left show the amount of missing data in the supermatrix for each OTU as a percentage of both the

(legend continued on next page)

M. mackini within Rhizaria does not appear to be an artifact of long branch attraction (LBA); when the 24 fastest-evolving taxa (other than *M. mackini*) were removed, the ML bootstrap support for *M. mackini* within Rhizaria increased from 91% to 100%, whereas the BI posterior probability remained at 1.0 (Figure 1).

To investigate the exact position of *M. mackini*, we used the more complex CATGTR + Γ4 model in combination with three smaller data sets focusing on Rhizaria (see the Supplemental Information): one retaining the broad rhizarian diversity (47 taxa; Table S2), one excluding the 10 fastest-evolving taxa (37 taxa; Table S2), and one excluding rhizarian taxa with more than 40% of missing positions (35 taxa; Table S2). These analyses were generally congruent with Figure 1: *M. mackini* branched with Retaria, *Gromia*, and *Corallomyxa* but did so with varying degrees of support (Figure S1). To further test this position, we used the approximately unbiased (AU) test to directly compare alternative topologies where *M. mackini* was placed on 12 different branches within Rhizaria (Supplemental Information). The alternatives where *M. mackini* was sister to *Gromia* and *Corallomyxa* ($p = 0.5$) or *Corallomyxa* alone ($p = 0.27$) were the only two topologies that were not rejected at the 5% threshold, which was in agreement with the signal recovered in the CAT analysis of the global data set (Figure 1). Finally, we tested the effect of heterotachy, a process that can impact phylogenetic reconstructions if the evolutionary rate is not constant across the tree [18]. When the covarion model was added to the CATGTR + Γ4 model, we found that the trees based on all reduced data sets converged to the same topology, wherein *M. mackini* is most closely related to Retaria (Figure S1).

Overall, our phylogenomic analysis unequivocally shows that *M. mackini* is a member of the supergroup Rhizaria, but its exact position within this group is less clear. Rhizaria have recently undergone substantial rearrangements, and new evidence has supported the Retaria hypothesis [19,20]. Retaria include two important groups of skeleton-building protists, Foraminifera and Radiolaria [21], and our results suggest that *M. mackini* may be closely related to this assemblage or its nearest sisters. However, the divergent nature of *M. mackini* sequences is problematic, as is the lack of genomic data from two rhizarian groups, Haplosporidia and Paramyxea. This is potentially significant, because both are rhizarian parasites of invertebrates, and Haplosporidia has been hypothesized to be related to Retaria [22, 23]. To evaluate the possibility that *M. mackini* is specifically related to Haplosporidia or Paramyxea, we inferred ML and BI phylogenies with the SSU rDNA gene. The paramyxean SSU rDNA is even more divergent than *M. mackini*, so it was removed from the alignment. Interestingly, the BI analysis recovered *M. mackini* within Haplosporidia (Figure S2). However, this position was not supported in the ML analysis, which showed that Haplosporidia was monophyletic to the exclusion of *M. mackini* (95%). Although the ML analysis did not rule out that *M. mackini* may be a sister to Haplosporidia, it is in general agreement with earlier reports proposing that *M. mackini* does not belong to this group [1,2].

Evidence for Mitochondrial-Derived Iron-Sulfur Cluster Formation in *Mikrocytos mackini*

The position of *M. mackini* within Rhizaria is significant because ultrastructural studies have failed to identify an organelle resembling a mitochondrion or MRO in this organism [1] and no other MRO-containing lineages are known in Rhizaria. If *M. mackini* contains a reduced organelle such as hydrogenosome or mitosome, then it must have evolved independently of other known MROs. Therefore, a comparison between its function and that of other MROs would provide important data as to how these organelles evolved. Mitochondria are sustained by hundreds of mitochondrion-encoded and -targeted proteins, which mostly function in oxidative phosphorylation, electron transport, the tricarboxylic acid cycle, and fatty acid metabolism as well as genome maintenance and expression [24–26]. MROs, on the other hand, have lost most or all of these functions. Only hydrogenosomes have retained ATP-producing pathways, whereas mitosomes have no obvious role in energy metabolism and have typically lost their genome entirely [27–35]; some organelles can take more intermediate forms with metabolic properties of aerobic and anaerobic mitochondria and of hydrogenosomes [36,37]. However, nearly all MROs have retained one function in common—the biosynthesis of iron-sulfur (Fe-S) clusters, which are essential for several cytosolic proteins [25,38,39].

To assess the fate of the *M. mackini* mitochondrion, we prefiltered our 15,874 contigs with CBOrg [40] in order to search for potential mitochondrion-derived genes. This approach resulted in 88 initial candidates, but, after manual inspection (see the Experimental Procedures), only four putative mitochondrial proteins remained (Figure 2): cysteine desulfurase (IscS), iron-sulfur cluster assembly enzyme (IscU), ferredoxin reductase (FdxR), and two copies of mitochondrial Hsp70 (mtHsp70). Phylogenetic analyses confirmed that IscS, IscU, FdxR, and one mtHsp70 (a534;558) clustered among mitochondrial and MRO copies and that they were not of host origin (Figure S3). However, the second mtHsp70 candidate (a827;2132) grouped with another *M. mackini* contig, and both branched with the cytosolic Hsp70 in other eukaryotes (Figure S3); therefore, this mtHsp70 candidate was not analyzed further. All four remaining proteins encoded an N-terminal extension predicted to be a mitochondrial-targeting transit peptide (Table 1), altogether showing that these proteins are derived from the mitochondrion and are most likely still targeted to and function in a mitochondrion-derived organelle.

The functional nature of these four mitochondrion-derived genes is significant. Genes relating to Fe-S cluster biosynthesis represent a tiny proportion of known mitochondrion-derived genes, and the greatest proportion of mitochondrial genes relate to classical energy-generation pathways, genome maintenance and expression, and other metabolic pathways. If our analysis had randomly identified a small fraction of *M. mackini* mitochondrial genes, then one would expect the genes to represent subsets of several pathways. Instead, all four identified genes participate in Fe-S cluster biosynthesis; i.e., IscS liberates sulfur from cysteine, which

missing amino acid positions (blue) and missing genes (yellow). Rhizaria are generally characterized by an elevated amount of missing data because only transcriptomes are available (*Bigelowiella natans* is the only complete genome). We designed our 119-gene supermatrix to minimize missing data for *M. mackini*. The impact of the missing data were evaluated with a reduced data set obtained by removing the 12 taxa with the most missing positions (35 OTU data set).

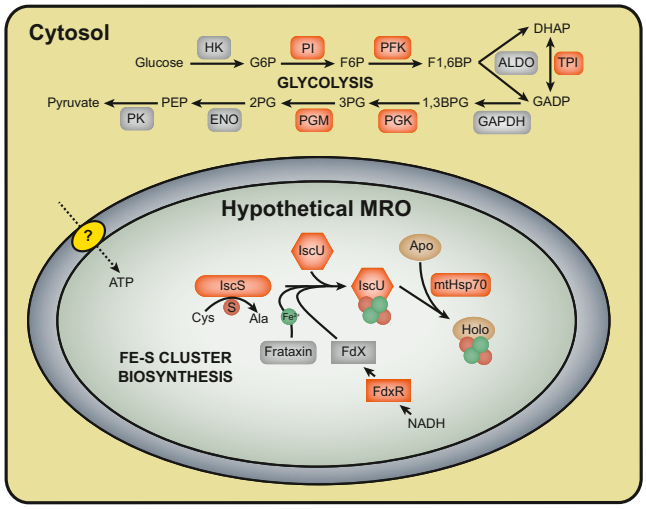

Figure 2. Hypothetical Summary of MRO Function in *M. mackini*

Genes identified in the transcriptome survey are shown in orange, and genes for proteins belonging to the same pathways that are missing from the data (but potentially present in *M. mackini*) are shown in gray. The four genes involved in Fe-S biosynthesis are mitochondrion-derived, encode N-terminal presequences resembling mitochondrial transit peptides, and are inferred to be targeted to an MRO. Genes for glycolytic enzymes are related to cytosolic homologs, do not encode leaders, and are inferred to be cytosolic. Proteins from the mitochondrial carrier family (MCF), including ATP transporters, were not identified in the data, but, given that ATP-dependent steps of Fe-S biosynthesis are represented, we speculate that some form of ATP transport is most likely present (denoted by a yellow circle). HK, hexokinase; G6P, glucose-6-phosphate; PI, phosphoglucose isomerase; F6P, fructose-6-phosphate; PFK, phosphofructokinase; F1,6BP, fructose-1,6-bisphosphate; ALDO, fructose bisphosphate aldolase; DHAP, dihydroxyacetone phosphate; GADP, glyceraldehyde-3-phosphate; TPI, triosephosphate isomerase; GAPDH, glyceraldehyde phosphate dehydrogenase; 1,3BPG, 1,3-bisphosphoglycerate; PGK, phosphoglycerate kinase; 3PG, 3-phsphoglycerate; 2PG, 2-phosphoglycerate; ENO, enolase; PEP, phosphoenolpyruvate; PK, pyruvate kinase; IscS, cysteine desulfurase; IscU, iron-sulfur cluster assembly enzyme; mtHsp70, chaperone protein DnaK; FdX ferredoxin; FdxR, ferredoxin reductase.

initiates the maturation of mitochondrial Fe-S proteins, and IscU binds the iron substrate for incorporation into the Fe-S scaffold [39]; furthermore, this process depends on FdxR, which possibly participates in the reduction of sulfur to sulphide, and mtHsp70, which binds the apoproteins to maintain their structure prior to transfer of the Fe-S cluster [38]. Other Fe-S biosynthetic proteins that were not recovered, such as ferredoxin or frataxin, may be truly lacking in *M. mackini* or just missing from our data (Figure 2). These core components of Fe-S cluster biosynthesis have been inferred or directly demonstrated to localize to mitosomes in the diplomonad *Giardia lamblia*, the microsporidians *Encephalitozoon cuniculi* and *Trachipleistophora hominis*, and the apicomplexan *Cryptosporidium parvum* [41–44]. Similarly, the hydrogenosome of the parabasalid *Trichomonas vaginalis* also contains core components of this pathways [28,45], as does the unusual MRO of *Blastocystis hominis* [36,46]. Uniquely, in *Entamoeba histolytica*, the Fe-S cluster machinery was replaced by bacterial-type components, which appear to localize to the cytoplasm rather than mitosomes (although this is still debated [47,48]), and the mitosome possesses a sulfate activation pathway [49]. However, in general, the most common functional hallmark of a mitosome is the retention of the Fe-S pathway and little or nothing else. It is formally possible that genes involved in energy-generating pathways were systematically not detected in the *M. mackini* transcriptome. But, given that we only identified genes involved in Fe-S biosynthesis out of the hundreds of possible mitochondrion-derived genes, we hypothesize that *M. mackini* most likely harbors a previously undescribed double-membrane-bounded mitosome-like organelle that does not function in energy generation (Figure 2).

If the *M. mackini* MRO is restricted to Fe-S biosynthesis, then questions arise about how *M. mackini* generates ATP and how its MRO could support energy-dependent reactions, such as the transfer and incorporation of the Fe-S cluster into recipient apoproteins by the mtHsp70 chaperone. One possibility is that energy generation is strictly cytosolic, such as in other mitosome-containing lineages. From our transcriptomic survey, we identified homologs of five *M. mackini* genes involved in glycolysis (Figure 2), a pathway commonly linked to some form of ATP-generating fermentation in other "amitochondriates" [25]. Alternatively, it is also conceivable that *M. mackini* steals ATP from its host. Interestingly, a very close association between *M. mackini* and host cell mitochondria has been observed, and it has even been proposed that mitochondrion-derived material passes from the host to the parasite [1]. This kind of association has also been reported in microsporidia, which sometimes recruit host mitochondria near their plasma membrane [50,51]. The microsporidian *E. cuniculi* is known to import ATP directly from its host through unusual transporters derived from bacterial intracellular parasites such as *Rickettsia* and *Chlamydia* [52]. None of these bacterial transporters were present in our transcriptomic survey. Indeed, we did not identify any members of the mitochondrial carrier family responsible for the import and export of metabolites, including ADP/ATP transporters that are necessary for importing ATP into mitosomes in order to satisfy ATP-dependent steps in Fe-S biosynthesis [29,52–55], leaving open the questions of if and how ATP is generated and transported in *M. mackini* (Figure 2).

In summary, we present robust evidence that the enigmatic microcell parasite *M. mackini* belongs to the eukaryotic supergroup Rhizaria and that it most likely harbors a reduced MRO. This represents the first evidence of an MRO-containing organism in this supergroup and, more generally, a new case

Table 1. Putative Mitochondrial Proteins in *M. mackini*

Gene Name	Product	ID	MITOPROT[a]	IPSORT[a]	PREDOTAR[a]	Figure
IscS	Cysteine desulfurase	a4960;323	M (0.99)	M	(M)	Figure S1A
IscU	Iron-sulfur cluster assembly enzyme	a2808;697	M (0.96)	M	M	Figure S1B
FdxR	Ferredoxin reductase	a2285;683	M (0.85)	M	(M)	Figure S1C
mtHsp70	Chaperone protein DnaK	a534;558	M (0.94)	—	—	Figure S1D

[a]MITOPROT, IPSORT, and PREDOTAR were used to predict the mitochondrial-targeting leader in the N-terminal extensions of *M. mackini* putative mitochondrial proteins. The dash indicates no typical targeting signal, and (M) means possibly mitochondrial.

of amitochondriate eukaryote. Despite serious technical challenges presented by a seasonal, tiny, and uncultivated intracellular parasite, a better understanding of the function of this MRO is now required. Transcriptome analysis suggests that it may be functionally limited to Fe-S biosynthesis, as in the case of mitosomes. To test this hypothesis, localizing proteins to the organelle and performing a proteomic assessment of its functions as well as obtaining a complete nuclear genome would all be highly informative. Extremely reductive evolution has driven the transition from mitochondria to MROs in other protistan parasites. We speculate that the MRO in *M. mackini* has converged toward a similar condition to that seen in microsporidia in response to adaptation to its intracellular lifestyle, leaving only the minimal toolkit necessary for maintaining the almost universal function of mitochondria in Fe-S cluster biosynthesis. Because this MRO represents a new instance of a very small set of known cases for studying the process of extreme mitochondrial reduction, further examination of the apparent parallel evolution of this organelle and how characteristics like protein targeting work will also be important additions to the field of mitochondrial evolution.

Experimental Procedures

The Supplemental Information details the complete experimental procedures. In summary, a concentrated and semipurified isolate of *M. mackini* was prepared from laboratory-infected Pacific oysters (*C. gigas*) according to previously published procedures [56]. Total RNA was isolated for the generation of complementary DNA, which was sequenced on an Illumina HiSeq 2000 system. The assembly of the raw reads and contamination removal produced 15,874 contigs with an average size of 501 bp. *M. mackini* was added to a set of more than 250 genes maintained up to date after performing a procedure that was previously described [10,15]. Genes for several rhizarian taxa were also added, including 13 unpublished genes (see Table S2 for the complete list of taxa). The final gene selection included 119 genes (23,162 aa) and 101 OTUs representative of all eukaryotic diversity. Also, three reduced data sets were analyzed (see the Supplemental Information). Each data set was analyzed by ML with RAxML v.7.2.8 [57] and the Bayesian approach with PhyloBayes v.3.3e and the MPI version 1.3b [58]. All ML analyses were performed with the LG + Γ4 model of evolution and 100 bootstrap replicates. The Bayesian analyses were done with the site-heterogeneous mixture CAT or CATGTR models + Γ4. Modeling of the heterotachy was added to the CATGTR + Γ4 model with the Tuffley and Steel covarion model [59]. Details of additional phylogenetic analyses, including the AU test and SSU rDNA tree reconstruction, can be found in the Supplemental Information. In order to detect proteins of mitochondrial origin, multiple BLASTX comparisons between the *M. mackini* data set and mitochondrial proteomes and subtractive databases from *Homo sapiens*, *Saccharomyces cerevisiae*, *T. vaginalis*, and *T. thermophila* were performed with CBOrg [40]. This procedure led to the identification of 88 candidates, which were then used as queries in BLASTX searches against the GenBank and Swissprot databases (e value $\leq 1\times10^{-10}$). Despite the initial filtering against host contamination (see the Supplemental Information), 32 candidates matched a molluscan sequence (mostly *C. gigas*) as best hits and were removed from the list. Another four sequences corresponded to prokaryotic contamination and were also removed. A close look at the remaining 52 proteins showed that, with the exception of ten proteins, most were likely not of mitochondrial function or not specific to mitochondria (Table S3). Among these, five proteins were discarded because phylogenetic trees revealed that they corresponded to metazoan sequences, although not to a specific taxon, or the open reading frame was too short to infer any origin (<43 aa). The final list of the four putative mitochondrial proteins identified from *M. mackini* in this study is shown in Tables 1 and S3.

Accession Numbers

The Transcriptome Shotgun Assembly project for *M. mackini* has been deposited in the DDBJ/EMBL GenBank under the accession number GAHX00000000. The version described in this paper is the first version, GAHX01000000. The new rhizarian sequences were deposited at the European Nucleotide Archive under the accession numbers HG316219–HG316452 and are directly available at http://www.ebi.ac.uk/ena/data/view/HG316219-HG316452.

Supplemental Information

Supplemental Information contains Supplemental Experimental Procedures, three figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2013.06.033.

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References

1. Hine, P.M., Bower, S.M., Meyer, G.R., Cochennec-Laureau, N., and Berthe, F.C. (2001). Ultrastructure of Mikrocytos mackini, the cause of Denman Island disease in oysters Crassostrea spp. and Ostrea spp. in British Columbia, Canada. Dis. Aquat. Organ. *45*, 215–227.
2. Carnegie, R.B., Meyer, G.R., Blackbourn, J., Cochennec-Laureau, N., Berthe, F.C.J., and Bower, S.M. (2003). Molecular detection of the oyster parasite Mikrocytos mackini, and a preliminary phylogenetic analysis. Dis. Aquat. Organ. *54*, 219–227.
3. Gagné, N., Cochennec, N., Stephenson, M., McGladdery, S., Meyer, G.R., and Bower, S.M. (2008). First report of a Mikrocytos-like parasite in European oysters Ostrea edulis from Canada after transport and quarantine in France. Dis. Aquat. Organ. *80*, 27–35.
4. Wang, Z., Liang, Y., and Lu, X. (2010). Use of histopathology, PCR and in situ hybridization methods to detect the parasite Mikrocytos sp. in Pacific oyster Crassostrea gigas from the northern coast of the Yellow Sea, China. Aquat. Living Resour. *23*, 125–130.
5. Abbott, C.L., Gilmore, S.R., Lowe, G., Meyer, G., and Bower, S. (2011). Sequence homogeneity of internal transcribed spacer rDNA in Mikrocytos mackini and detection of Mikrocytos sp. in a new location. Dis. Aquat. Organ. *93*, 243–250.
6. Carnegie, R.B., and Cochennec-Laureau, N. (2004). Microcell parasites of oysters: Recent insights and future trends. Aquat. Living Resour. *17*, 519–528.
7. Hjort, K., Goldberg, A.V., Tsaousis, A.D., Hirt, R.P., and Embley, T.M. (2010). Diversity and reductive evolution of mitochondria among microbial eukaryotes. Philos. Trans. R. Soc. Lond. B Biol. Sci. *365*, 713–727.
8. Shiflett, A.M., and Johnson, P.J. (2010). Mitochondrion-related organelles in eukaryotic protists. Annu. Rev. Microbiol. *64*, 409–429.
9. Hervio, D., Bower, S.M., and Meyer, G.R. (1996). Detection, isolation, and experimental transmission of Mikrocytos mackini, a microcell parasite of Pacific oysters Crassostrea gigas (Thunberg). J. Invertebr. Pathol. *67*, 72–79.
10. Burki, F., Okamoto, N., Pombert, J.-F.F., and Keeling, P.J. (2012). The evolutionary history of haptophytes and cryptophytes: phylogenomic evidence for separate origins. Proc. Biol. Sci. *279*, 2246–2254.
11. Burki, F., Shalchian-Tabrizi, K., Minge, M., Skjaeveland, A., Nikolaev, S.I., Jakobsen, K.S., and Pawlowski, J. (2007). Phylogenomics reshuffles the eukaryotic supergroups. PLoS ONE *2*, e790.
12. Hampl, V., Hug, L., Leigh, J.W., Dacks, J.B., Lang, B.F., Simpson, A.G.B., and Roger, A.J. (2009). Phylogenomic analyses support the monophyly of Excavata and resolve relationships among eukaryotic "supergroups". Proc. Natl. Acad. Sci. USA *106*, 3859–3864.

13. Zhao, S., Burki, F., Bråte, J., Keeling, P.J., Klaveness, D., and Shalchian-Tabrizi, K. (2012). Collodictyon—an ancient lineage in the tree of eukaryotes. Mol. Biol. Evol. *29*, 1557–1568.
14. Rodríguez-Ezpeleta, N., Brinkmann, H., Burger, G., Roger, A.J., Gray, M.W., Philippe, H.H., and Lang, B.F. (2007). Toward resolving the eukaryotic tree: the phylogenetic positions of jakobids and cercozoans. Curr. Biol. *17*, 1420–1425.
15. Burki, F., Inagaki, Y., Bråte, J., Archibald, J.M., Keeling, P.J., Cavalier-Smith, T.T., Sakaguchi, M., Hashimoto, T., Horak, A., Kumar, S., et al. (2009). Large-scale phylogenomic analyses reveal that two enigmatic protist lineages, telonemia and centroheliozoa, are related to photosynthetic chromalveolates. Genome Biol. Evol. *1*, 231–238.
16. Brown, M.W., Kolisko, M., Silberman, J.D., and Roger, A.J. (2012). Aggregative multicellularity evolved independently in the eukaryotic supergroup Rhizaria. Curr. Biol. *22*, 1123–1127.
17. Derelle, R., and Lang, B.F. (2012). Rooting the eukaryotic tree with mitochondrial and bacterial proteins. Mol. Biol. Evol. *29*, 1277–1289.
18. Lopez, P., Casane, D., and Philippe, H.H. (2002). Heterotachy, an important process of protein evolution. Mol. Biol. Evol. *19*, 1–7.
19. Burki, F., Kudryavtsev, A., Matz, M.V., Aglyamova, G.V., Bulman, S., Fiers, M., Keeling, P.J., and Pawlowski, J. (2010). Evolution of Rhizaria: new insights from phylogenomic analysis of uncultivated protists. BMC Evol. Biol. *10*, 377.
20. Sierra, R., Matz, M.V., Aglyamova, G., Pillet, L., Decelle, J., Not, F.F., de Vargas, C.C., and Pawlowski, J. (2013). Deep relationships of Rhizaria revealed by phylogenomics: a farewell to Haeckel's Radiolaria. Mol. Phylogenet. Evol. *67*, 53–59.
21. Moreira, D., von der Heyden, S., Bass, D., López-García, P., Chao, E., and Cavalier-Smith, T. (2007). Global eukaryote phylogeny: Combined small- and large-subunit ribosomal DNA trees support monophyly of Rhizaria, Retaria and Excavata. Mol. Phylogenet. Evol. *44*, 255–266.
22. Tekle, Y.I., Grant, J., Cole, J.C., Nerad, T.A., Anderson, O.R., Patterson, D.J., and Katz, L.A. (2007). A multigene analysis of Corallomyxa tenera sp. nov. suggests its membership in a clade that includes Gromia, Haplosporidia and Foraminifera. Protist *158*, 457–472.
23. Pawlowski, J., and Burki, F. (2009). Untangling the phylogeny of amoeboid protists. J. Eukaryot. Microbiol. *56*, 16–25.
24. Gray, M.W., Lang, B.F., and Burger, G. (2004). Mitochondria of protists. Annu. Rev. Genet. *38*, 477–524.
25. Müller, M., Mentel, M., van Hellemond, J.J., Henze, K., Woehle, C., Gould, S.B., Yu, R.Y., van der Giezen, M., Tielens, A.G.M., and Martin, W.F. (2012). Biochemistry and evolution of anaerobic energy metabolism in eukaryotes. Microbiol. Mol. Biol. Rev. *76*, 444–495.
26. Embley, T.M., and Martin, W.F. (2006). Eukaryotic evolution, changes and challenges. Nature *440*, 623–630.
27. Müller, M. (1993). The hydrogenosome. J. Gen. Microbiol. *139*, 2879–2889.
28. Hrdy, I., Tachezy, J., and Müller, M. (2008). Metabolism of the Trichomonad hydrogenosome J. Tachezy.
29. Carlton, J.M.J., Hirt, R.P.R., Silva, J.C.J., Delcher, A.L.A., Schatz, M.M., Zhao, Q.Q., Wortman, J.R.J., Bidwell, S.L.S., Alsmark, U.C.M.U., Besteiro, S.S., et al. (2007). Draft genome sequence of the sexually transmitted pathogen Trichomonas vaginalis. Science *315*, 207–212.
30. Tovar, J., León-Avila, G., Sánchez, L.B., Sutak, R., Tachezy, J., van der Giezen, M., Hernández, M., Müller, M., and Lucocq, J.M. (2003). Mitochondrial remnant organelles of Giardia function in iron-sulphur protein maturation. Nature *426*, 172–176.
31. Tovar, J., Fischer, A., and Clark, C.G. (1999). The mitosome, a novel organelle related to mitochondria in the amitochondrial parasite Entamoeba histolytica. Mol. Microbiol. *32*, 1013–1021.
32. Williams, B.A.P.B., Hirt, R.P., Lucocq, J.M., and Embley, T.M. (2002). A mitochondrial remnant in the microsporidian Trachipleistophora hominis. Nature *418*, 865–869.
33. Hampl, V., Silberman, J.D., Stechmann, A., Diaz-Triviño, S., Johnson, P.J., and Roger, A.J. (2008). Genetic evidence for a mitochondriate ancestry in the 'amitochondriate' flagellate Trimastix pyriformis. PLoS ONE *3*, e1383–e1383.
34. Abrahamsen, M.S., Templeton, T.J., Enomoto, S., Abrahante, J.E., Zhu, G., Lancto, C.A., Deng, M., Liu, C., Widmer, G., Tzipori, S., et al. (2004). Complete genome sequence of the apicomplexan, Cryptosporidium parvum. Science *304*, 441–445.
35. Yarlett, N.N., Orpin, C.G.C., Munn, E.A.E., Yarlett, N.C.N., and Greenwood, C.A.C. (1986). Hydrogenosomes in the rumen fungus Neocallimastix patriciarum. Biochem. J. *236*, 729–739.
36. Stechmann, A., Hamblin, K., Pérez-Brocal, V., Gaston, D.D., Richmond, G.S., van der Giezen, M., Clark, C.G., and Roger, A.J. (2008). Organelles in Blastocystis that blur the distinction between mitochondria and hydrogenosomes. Curr. Biol. *18*, 580–585.
37. Boxma, B., de Graaf, R.M., van der Staay, G.W.M., van Alen, T.A., Ricard, G., Gabaldón, T., van Hoek, A.H.A.M., Moon-van der Staay, S.Y., Koopman, W.J.H., van Hellemond, J.J., et al. (2005). An anaerobic mitochondrion that produces hydrogen. Nature *434*, 74–79.
38. Lill, R. (2009). Function and biogenesis of iron-sulphur proteins. Nature *460*, 831–838.
39. Lill, R., and Mühlenhoff, U. (2008). Maturation of iron-sulfur proteins in eukaryotes: mechanisms, connected processes, and diseases. Annu. Rev. Biochem. *77*, 669–700.
40. Gaston, D.D., Tsaousis, A.D., and Roger, A.J. (2009). Predicting proteomes of mitochondria and related organelles from genomic and expressed sequence tag data. Methods Enzymol. *457*, 21–47.
41. Goldberg, A.V., Molik, S., Tsaousis, A.D., Neumann, K., Kuhnke, G., Delbac, F., Vivarès, C.P., Hirt, R.P., Lill, R., and Embley, T.M. (2008). Localization and functionality of microsporidian iron-sulphur cluster assembly proteins. Nature *452*, 624–628.
42. LaGier, M.J., Tachezy, J., Stejskal, F., Kutisova, K., and Keithly, J.S. (2003). Mitochondrial-type iron-sulfur cluster biosynthesis genes (IscS and IscU) in the apicomplexan Cryptosporidium parvum. Microbiology *149*, 3519–3530.
43. Slapeta, J., and Keithly, J.S. (2004). Cryptosporidium parvum mitochondrial-type HSP70 targets homologous and heterologous mitochondria. Eukaryot. Cell *3*, 483–494.
44. Williams, B.A.P., Cali, A., Takvorian, P.M., and Keeling, P.J. (2008). Distinct localization patterns of two putative mitochondrial proteins in the microsporidian Encephalitozoon cuniculi. J. Eukaryot. Microbiol. *55*, 131–133.
45. Sutak, R., Dolezal, P., Fiumera, H.L., Hrdy, I., Dancis, A., Delgadillo-Correa, M., Johnson, P.J., Müller, M., and Tachezy, J. (2004). Mitochondrial-type assembly of FeS centers in the hydrogenosomes of the amitochondriate eukaryote Trichomonas vaginalis. Proc. Natl. Acad. Sci. USA *101*, 10368–10373.
46. Tsaousis, A.D.A., Ollagnier de Choudens, S., Gentekaki, E.E., Long, S.S., Gaston, D.D., Stechmann, A.A., Vinella, D.D., Py, B.B., Fontecave, M.M., Barras, F.F., et al. (2012). Evolution of Fe/S cluster biogenesis in the anaerobic parasite Blastocystis. Proc. Natl. Acad. Sci. USA *109*, 10426–10431.
47. Maralikova, B., Ali, V., Nakada-Tsukui, K., Nozaki, T., van der Giezen, M., Henze, K., and Tovar, J. (2010). Bacterial-type oxygen detoxification and iron-sulfur cluster assembly in amoebal relict mitochondria. Cell. Microbiol. *12*, 331–342.
48. Nývltová, E., Šuták, R., Harant, K., Šedinová, M., Hrdy, I., Paces, J., Vlček, C., and Tachezy, J. (2013). NIF-type iron-sulfur cluster assembly system is duplicated and distributed in the mitochondria and cytosol of Mastigamoeba balamuthi. Proc. Natl. Acad. Sci. USA *110*, 7371–7376.
49. Mi-ichi, F., Abu Yousuf, M., Nakada-Tsukui, K., and Nozaki, T. (2009). Mitosomes in Entamoeba histolytica contain a sulfate activation pathway. Proc. Natl. Acad. Sci. USA *106*, 21731–21736.
50. Scanlon, M., Leitch, G.J., Visvesvara, G.S., and Shaw, A.P. (2004). Relationship between the host cell mitochondria and the parasitophorous vacuole in cells infected with Encephalitozoon microsporidia. J. Eukaryot. Microbiol. *51*, 81–87.
51. Williams, B.A.P. (2009). Unique physiology of host-parasite interactions in microsporidia infections. Cell. Microbiol. *11*, 1551–1560.
52. Tsaousis, A.D., Kunji, E.R.S., Goldberg, A.V., Lucocq, J.M., Hirt, R.P., and Embley, T.M. (2008). A novel route for ATP acquisition by the remnant mitochondria of Encephalitozoon cuniculi. Nature *453*, 553–556.
53. Loftus, B.B., Anderson, I.I., Davies, R.R., Alsmark, U.C.M.U., Samuelson, J.J., Amedeo, P.P., Roncaglia, P.P., Berriman, M.M., Hirt, R.P.R., Mann, B.J.B., et al. (2005). The genome of the protist parasite Entamoeba histolytica. Nature *433*, 865–868.
54. Kunji, E.R.S. (2004). The role and structure of mitochondrial carriers. FEBS Lett. *564*, 239–244.
55. Williams, B.A.P.B., Haferkamp, I.I., and Keeling, P.J. (2008). An ADP/ATP-specific mitochondrial carrier protein in the microsporidian Antonospora locustae. J. Mol. Biol. *375*, 1249–1257.
56. Joly, J.-P., Bower, S.M., and Meyer, G.R. (2001). A simple technique to concentrate the protozoan Mikrocytos mackini, causative agent of Denman Island Disease in oysters. J. Parasitol. *87*, 432–434.

57. Stamatakis, A. (2006). RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics *22*, 2688–2690.
58. Lartillot, N., Lepage, T., and Blanquart, S. (2009). PhyloBayes 3: a Bayesian software package for phylogenetic reconstruction and molecular dating. Bioinformatics *25*, 2286–2288.
59. Tuffley, C., and Steel, M. (1998). Modeling the covarion hypothesis of nucleotide substitution. Math. Biosci. *147*, 63–91.