Multiple Independent Origins of Apicomplexan-Like Parasites

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SUMMARY

The apicomplexans are a group of obligate animal pathogens that include Plasmodium (malaria), Toxoplasma (toxoplasmosis), and Cryptosporidium (cryptosporidiosis) [1]. They are an extremely diverse and specious group but are nevertheless united by a distinctive suite of cytoskeletal and secretory structures related to infection, called the apical complex, which is used to recognize and gain entry into animal host cells. The apicomplexans are also known to have evolved from free-living photosynthetic ancestors and retain a relict plastid (the apicoplast), which is non-photosynthetic but houses a number of other essential metabolic pathways [2]. Their closest relatives include a mix of both photosynthetic algae (chromerids) and non-photosynthetic microbial predators (colpodellids) [3]. Genomic analyses of these free-living relatives have revealed a great deal about how the alga-parasite transition may have taken place, as well as origins of parasitism more generally [4]. Here, we show that, despite the surprisingly complex origin of apicomplexans from algae, this transition actually occurred at least three times independently. Using single-cell genomics and transcriptomics from diverse uncultivated parasites, we find that two genera previously classified within the Apicomplexa, Piridium and Platyproteum, form separately branching lineages in phylogenomic analyses. Both retain cryptic plastids with genomic and metabolic features convergent with apicomplexans. These findings suggest a predilection in this lineage for both the convergent loss of photosynthesis and transition to parasitism, resulting in multiple lineages of superficially similar animal parasites.

RESULTS AND DISCUSSION

To gain a deeper understanding of the origin of parasitism in apicomplexans, we used single-cell sequencing to characterize the genomes and transcriptomes from a number of uncultivated parasites representing poorly studied lineages of apicomplexans. Specifically, we generated transcriptome data from individual trophozoite cells of the gregarine apicomplexans Monocystis agilis, Lecudina tuzetae, Pterospora schizosoma, Heliospora capraellae, and Platyproteum sp., using single cells documented microscopically and manually isolated directly from their animal hosts (Figures 1A–1E). In addition, we generated both genomic and transcriptomic data from gamogonic stages of Piridium sociabile, an apicomplexan isolated from the foot tissue cells of the common marine whelk, Buccinum undatum (Figure 1F). These gregarines represent subgroups of both marine (Pterospora, Heliospora, Lecudina, and Platyproteum) and terrestrial (Monocystis) parasites, and the limited available molecular data (from small subunit [SSU] rRNA) are divergent but generally show them to be diverse, early branching apicomplexans [5–8] (Figure S1). Platyproteum was the most recently described by detailed microscopy and molecular phylogenetic analyses using SSU rDNA sequences; these data suggest that it is a particularly deep-branched apicomplexan [9, 10]. Piridium sociabile is even more poorly studied: found in 1932 as an intracellular infection and was morphologically classified as a schizogregarine [11].

The relationships of these six taxa to the Apicomplexa were examined by phylogenomics using a concatenated alignment of 39 taxa and 189 nucleus-encoded proteins that have been previously used in in both eukaryote-wide and phylum-level phylogenies [12, 13]. Their positions in the resulting tree are strongly and consistently resolved by both maximum likelihood (C40+LG+I+F model) and Bayesian (CAT-GTR) analyses (Figure 1G). Surprisingly, the phylogeny shows that neither Piridium nor Platyproteum branch within the Apicomplexa. Instead, Piridium branches within the sister group to the Apicomplexa, the “chromodellids” (chromerids + colpodellids), with complete support as sister to the photosynthetic alga Vitrella brassiciformis. Platyproteum forms a new lineage, also with complete
support, sister to the clade consisting of apicomplexans and chrompodellids collectively. The four more canonical gregarines (Monocystis, Lecudina, Pterospora, and Heliospora) formed a monophyletic group of deep-branching apicomplexans that interestingly excludes Cryptosporidium. This robust phylogeny not only confirms that photosynthesis was lost multiple times independently around the origin of the Apicomplexa but more surprisingly shows that the highly derived mode of animal parasitism that is characteristic of the Apicomplexa also arose multiple times independently.

To further investigate the convergent evolution of parasitic lifestyles in Piridium and Platyproteum, we examined plastid retention and function, a well-studied trait of the Apicomplexa [2, 3]. With both genomic and transcriptomic data from Piridium, we first assembled its complete plastid genome (Figures 2A and S2), which is strikingly similar in size, architecture, and gene content to apicoplast genomes (Figure 2B). The Piridium plastid genome is a highly reduced compact circle (~34 kb) with all remaining genes in perfect synteny with homologs in its closest relative, the photosynthetic Vitrella. Similar to the apicoplast, it is extremely AT rich (21% G+C content) and uses a non-canonical genetic code where UGA encodes tryptophan (as seen in Chromera, Toxoplasma, and corallicolids, but not in the more closely related Vitrella) [14, 15]. It retains similar ribosomal genes as well as the same bacterial RNA polymerases (rpoB, rpoC1, and rpoC2) and other protein-coding genes (sufA, clpC, and tufA) as apicoplasts. It has also convergently lost all genes relating to photosynthesis, as well as rps18, rpl13, rpl27, secA, and secY (Figure 2C). Reflecting its origin from a chrompodellid ancestor, the Piridium plastid also encodes a handful of genes that are present in Vitrella but absent from apicoplasts: rps14; rpl3; and rpoA. Curiously, only a partial rRNA inverted repeat remains in Piridium; this repeat is ancestral to all apicomplexans and chrompodellids but has also similarly been lost in the piroplasm apicomplexans, Babesia and Theileria [16, 17].

Apicomplexans depend on apicoplasts for essential biosynthesis of four compounds: isoprenoids (the non-mevalonate pathway); heme; iron-sulfur (Fe-S) clusters; and fatty acids (the type II fatty acid pathway) [2]. All apicomplexans rely on these pathways except piroplasms, which have lost the FASII and heme pathway and use cytosolic FASI instead, and Cryptosporidium, which can salvage the metabolites from its host and has lost its plastid entirely [18, 19]. We identified all enzymes from these pathways and all enzymes for analogous and homologous cytosolic pathways using profile hidden Markov models (HMMs) and analyzed the resulting genes for evidence of distinctive N-terminal bipartite plastid-targeting peptides (Figure 2C; Table S1). It is impossible to conclude that any single gene is absent based on transcriptomic data alone, so only the absence of all genes for entire biochemical pathways is considered here. The
dependency on plastid metabolism in *Piridium* is identical to most apicomplexans, with the retention of all four pathways but no photosystems or other known plastid functions. *Platyproteum* is similar but has also lost the FASII pathway and so more resembles the piroplasms [17, 18].

Interestingly, the same analysis on the clade of gregarines revealed a greater degree of variation from other apicomplexans than seen in the cryptic plastids that evolved in parallel (Figure 2A). Like *Cryptosporidium*, the terrestrial gregarines *Monocystis* and *Gregarina* have completely lost all plastid metabolism and likely also lost the organelle (which also suggests that the phylogenetic relationship between *Cryptosporidium* and terrestrial gregarines remains uncertain) [19, 20]. In contrast, however, the marine gregarines *Lecudina* and *Pterospora* retain the complete FASII pathway but no other identifiable plastid metabolism. This is the first evidence of a plastid in any gregarine and is also functionally curious, because it is isoprenoid biosynthesis that has been proposed to be the main barrier to plastid loss [3].

The gregarines thus suggest that plastid dependency is highly context dependent.

Looking beyond the plastid, metabolic reconstructions based on KEGG (Kyoto Encyclopedia of Genes and Genomes) identifiers across the whole genome confirm an overall convergence of functional reduction but also some divergence (Figure 3). Both *Piridium* and *Platyproteum* have, as expected, substantially reduced their metabolic functions compared to their free-living chrompodellid relatives. However, neither is as reduced as apicomplexan parasites. In both cases, a few core pathways, such as the glyoxylate cycle and pyrimidine catabolism, have been retained (Table S2). Of the two, *Piridium* contains the greatest breadth of biosynthetic functions that were mostly lost in all other parasitic groups, such as *de novo* amino acid biosynthesis (isoleucine and arginine) and purine biosynthesis (inosine) and degradation. Surprisingly, the gregarine *Monocystis agilis* has also retained a greater metabolic capacity than other apicomplexans. Although its greater functional capacity relative to other gregarines may be due to better sequencing coverage, the majority of other apicomplexans are reconstructed from whole genomes, suggesting that the baseline metabolic complexity of the group as a whole is greater than was previously thought.

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**Figure 2. Plastid Dependency in *Piridium* and *Platyproteum* Has Evolved Convergently to Apicomplexans**

(A) Complete annotated plastid genome of *P. sociabile*.

(B) Presence of plastid biosynthetic pathways across the tree of apicomplexans and chrompodellids. Portions of the circles represent the proteins found in each pathway found (key shown on right). Black circles indicate the presence of complete N-terminal bipartite plastid-targeting peptides (only shown for newly added transcriptomes).

(C) Plastid gene content of apicomplexans and *Vitrella* (free-living, photosynthetic) compared to *Piridium*.

See also Table S1 and Figure S2.
The origin of apicomplexan parasites from free-living photosynthetic alga represents a major evolutionary transition between two very different modes of living, so different in this case that the idea was originally met with skepticism. The current data show that, however dramatic this transition may seem, it was not unique but rather repeated at least three times in related lineages of photosynthetic algae. The details of the parasitic machinery in *Piridium* and *Platyproteum* are unknown, so how detailed the convergence of their parasitic lifestyles may be will require more information, but they superficially resemble apicomplexans to the extent that they were classified within the group when formally described. The genomic and transcriptomic data presented here also suggest that the ancestors of these lineages maintained high levels of redundancy in metabolic pathways between compartments that persisted over long periods of evolutionary time and apparently shared some predilection to animal parasitism. The underlying reason for this is not clear, because the evolution of apicomplexan parasitism is not linked to the acquisition of any novel feature or machinery but is instead marked by loss and tinkering of the existing genomic repertoire.

**STAR + METHODS**

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**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2019.07.019.

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**AUTHOR CONTRIBUTIONS**


**DECLARATION OF INTERESTS**

The authors declare no competing interests.

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**REFERENCES**

### Star Methods

#### Key Resources Table

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**LEAD CONTACT AND MATERIALS AVAILABILITY**

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Varsha Mathur (varsha.mathur@botany.ubc.ca). This study did not generate new unique reagents.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

*Monocystis agilis* was isolated from the seminiferous vesicles of earthworms (*Lumbricus terrestris*), purchased from Berry’s Bait and Tackle, Richmond, British Columbia, Canada in November 2017. *Platyproteum* sp. was isolated from the gut of a peanut worm (*Sipuncula*) that was collected from Sint Joris Bay, Curacao in April 2018. *Lecudina tuzetae* and *Heliospora caprellae* were isolated...
from the guts of the animals Platynereis bicanaliculata, and Caprella californica respectively. They were both collected at low tide from Calvert Island, BC, Canada in June 2018. Pterospora schizosoma was isolated from the gut of a bamboo worm, Axiothella rubrocincta, that was collected from Friday Harbour, Washington, USA in June 2018. All of these parasites were collected in the trophozoite life stage (large feeding cells). Trophozoites were released into autoclaved filtered seawater by teasing apart the intestines/seminal vesicles of the respective hosts with pointed forceps.

*Piridium sociabile* was isolated from the common whelk, Buccinum undatum, that was collected using dredges across Breidafjördur, west coast of Iceland (65° 7.576’N; 22° 44.738’W). Whelks were sedated using 0.1% MgSO4 in seawater for 1-2 hours, then examined for the presence of *Piridium* cysts on the surface of the foot using a dissection microscope. Mature (large) cysts were gently squeezed with pointed forceps until the *Piridium* gamonts were released. The resulting exudate was collected into concave glass spot plates containing filtered seawater and rinsed with autoclaved seawater three times to remove host tissues and mucous.

**METHOD DETAILS**

**Genomics and transcriptomics of Piridium sociabile**

DNA and RNA from the resulting gamonts was then extracted using a QIAGEN, Allprep DNA/RNA Mini Kit (Cat. No. 80204). cDNA was synthesized using the SMARTseq2 protocol with seven cDNA amplification cycles [22]. RNA and DNA sequencing libraries were both prepared using Illumina Nexterra XT and Nextera protocol respectively, and sequenced using 2 × 300bp Illumina MiSeq (DNA) and 2x100bp Illumina HiSeq 2000 run (RNA). Both RNA and DNA reads were adaptor and quality trimmed with Trimomatic [23]. RNA reads were further processed to remove low complexity regions using PRINTseq [25] and were assembled into transcripts using Trinity v2.4 (with default settings) and translated into protein sequences using Transdecoder v.5 [24, 26].

**Genome assembly and annotation of Piridium sociabile**

The MIRA4 assembler was used to assemble the genomic DNA reads, which led to the assembly of single circular plastid genome chromosome [27]. This assembly was validated by mapping of the reads back to the assembly by Bowtie2 [28]. The plastid genome was then automatically annotated using MFAnnot (http://megasun.bch.umontreal.ca/cgi-bin/mfannot/mfannotInterface.pl) and RNAsweasel (http://megasun.bch.umontreal.ca/cgi-bin/RNAsweasel/RNAsweaselInterface.pl), followed by manual corrections in Geneious v11.1.5 (https://www.geneious.com).

**Transcriptomics of the gregarines and Platyproteum**

The single-cell trophozoites were washed at least three times in autoclaved filtered seawater, or ultrapure water (for *Monocystis*) and viewed and photographed under a Leica DMIL LED microscope equipped with a 40 × objective and a Sony α6000 camera. Single trophozoite cells were picked using a glass capillary micropipettes and transferred to a 0.2 mL thin-walled PCR tube containing 2 μL of cell lysis buffer (0.2% Triton X-100 and RNase inhibitor (Invitrogen)). cDNA was synthetized from the single cell, or a pool of 2-3 cells, using the Smart-Seq2 protocol [22]. The cDNA concentration was quantified on a Qubit 2.0 Fluorometer (Thermo Fisher Scientific Inc.).

Prior to high-throughput sequencing, 1μL of the final cDNA product was used as a template for a PCR amplification of the V4 region of the 18S rRNA gene using Phusion High-Fidelity DNA Polymerase (New England Biolabs, Thermo Scientific) and the general eukaryotic primer pair TAREuk454FWD1 and TAREukREV3 [21]. The PCR product was then sequenced by Sanger dideoxy sequencing. The SSU rRNA gene sequences were used to confirm the identity of the newly collected organisms and avoid animal host contamination using BLASTn to look for similar sequences in the non-redundant NCBI database [34]. Once the identity of the parasite was confirmed, sequencing libraries were prepared using the Nexterra XT protocol, and sequenced on a single lane of Illumina MiSeq using 250 bp paired end reads.

**Transcriptome assembly and annotation of the gregarines and Platyproteum**

The raw Illumina sequencing reads were merged using PEAR v0.9.6, and FastQC was used to assess the quality of the paired reads [32, 33]. The adaptor and primer sequences were trimmed using Trimmomatic v0.36 and the transcriptomes were assembled with Trinity v2.4.0 [23, 24]. The contigs were then filtered for animal host contaminants using BlobTools in addition to blastn and blastx searches against the NCBI nt database and the Swiss-Prot database, respectively [30, 34]. Coding sequences were predicted using a combination of TransDecoder v3.0.1 and similarity searches against the Swiss-Prot database [26]. Assessment of the quality of the assembly and annotation of the transcriptomes (including *Piridium*) was carried out using BUSCO [31].

**Ortholog identification, gene concatenation and phylogenomics**

In addition to our newly generated transcriptomes, the following transcriptomes and genomes were downloaded from EuPathDB and screened for orthologs; *Hammondia hammondi*, *Sarcocystis neurona*, *Eimeria falciformis*, and *Gregarina niphandrodes* [49]. All transcriptomes were comprehensively searched for a set of 263 genes that have been used in previous phylogenomic analyses [12, 13]. All the sequences in the 263 gene-set, representing a wide range of eukaryotes, were used as queries to search the above datasets using BLASTn [34]. The hits were then filtered using an e-value threshold of 1e-20 and a query coverage of 50%. Each of the gene-sets was then aligned using MAFFT L-INS-i v7.222 and trimmed using trimAI v1.2 with a gap-threshold of 80% [35, 36]. Single gene
trees were then constructed to identify paralogs and contaminants using IQ-TREE v.1.6.9 (LG+G4 model) or RAxML v8.2.12 (PROTGAMMALG model) with support from 1000 bootstraps [37, 38]. The resulting trees were manually scanned in FigTree v1.4.2 and contaminants and paralogous sequences were identified and removed [39]. The final cleaned gene-sets were filtered so that they contained only a maximum of 40% missing OTUs and then concatenated in SCaFoS v1.2.5 [29]. The resulting concatenated alignment consisted of 198 genes spanning 58,116 amino acid positions from 39 taxa. The phylogenomic maximum likelihood tree was constructed with the heterogenous mixture C40+LG+Γ4+F model as implemented in IQ-TREE (model LG+Γ4+F yielded identical topology) [38]. Statistical support was inferred using 500 bootstrap replicates using the LG+C40+Γ4+F PMSF profiles, and 1000 bootstrap replicates using the LG+Γ4+F model in RAxML [37, 50]. The Bayesian tree was computed using Phylobayes [40] under the GTR-CAT model with constant sites removed from the analyses. Four independent chain were run for 9 thousand generations and converged with maxdiff = 0.19 (20% burning) (Figure 1G).

For the plastid based phylogenomic analyses, a previously published dataset [51] was used and enriched with proteins from wide sets of publicly available apicoplast proteins and the *Piridium* plastid genome. The plastid phylogenomic tree was constructed using a concatenated alignment of 62 plastid-encoded proteins with RAxML using the LG+Γ4+F substitution model with 500 bootstrap replicates [37] (Figure S2).

**Search and identification of plastid proteins**

Profile hidden Markov models (HMMs) were used to identify plastid metabolic proteins in our transcriptomes based on curated alignments. To construct the curated alignments, known dinoflagellate proteins were used as queries in a BLASTp search (e-value threshold of 1e-5) against a comprehensive custom database containing representatives comprised of major eukaryotic groups, with a focus on plastid-containing lineages (dinoflagellates, chromophodellids, Apicomplexa, cryptophytes, haptophytes, stramenopiles, Archaeplastida) as well as selected taxa from non-plastid lineages (Opisthokonta, Amoebozoa, Apusozoa, Ancyromonadida and ciliates) and RefSeq data from all bacterial phyla at NCBI (https://www.ncbi.nlm.nih.gov/).

Last accessed December 2017) [34]. The database was subjected to CD-HIT with a similarity threshold of 85% to reduce redundant sequences and paralogs [41]. Results from blast searches were parsed for hits with a minimum query coverage of 50% and e-values of less than 1e-25 (or 1e-5 for HemD). The number of bacterial hits was restrained to 20 hits per phylum (for FCB group, most classes of Proteobacteria, PVC group, Spirochaetes, Actinobacteria, Cyanobacteria (unranked) and Firmicutes) or 10 per phylum (remaining bacterial phyla) as defined by NCBI taxonomy. Parse hits were aligned with MAFFT v. 7.212, using the–auto option, poorly aligned regions were eliminated using trimAl v.1.2 with a gap threshold of 80% [35, 36]. Maximum likelihood tree reconstructions were then performed with FastTree v. 2.1.7 using the default options [42]. The resulting phylogenies and underlying alignments were inspected manually to remove contaminations, recent paralogs and duplicate sequences. The cleaned, unaligned sequences were then subjected to filtering with PREQUAL using the default options to remove nonhomologous residues introduced by poor-quality sequences, followed by alignment with MAFFT GINSi using the VSM option (unalignlevel 0.6) to control over-alignment [36, 43]. The alignments were subjected to Divvier (https://github.com/simonwhelan/Divvier) using the divvygap option to improve homology inference before removing ambiguously aligned sites with trimAl v. 1.2 (gap threshold of 1%) [35]. Trees for final sequence curation were calculated with IQ-TREE v. 1.6.5, using the mset option to restrict model selection (to DAYHOFF, DCMUT, JTT, WAG, VT, BLOSUM62, LG, PMB, JTTDCMUT) for ModelFinder, while branch support was assessed with 1000 ultrafast bootstrap replicates, and once more subjected to manual inspection [38, 52].

Profile HMMs were then generated using these curated alignments and HMM searches were conducted on all transcriptomes and genomes using HMMER v3.1 and an e-value threshold of 1e-5 [53]. All the hits were then extracted and incorporated into the original alignments and realigned using MAFFT v7.222 (-auto option). The resulting alignments were then used to generate phylogenies in IQ-TREE v.1.6.9 using the LG+F+G4 substitution model and statistical support was assessed using 1000 ultrafast bootstrap replicates [38, 52]. The phylogenies were then manually scanned in FigTree v1.4.2 and contaminants, paralogs, mitochondrial sequences, and long-branching divergent sequences were identified and removed [39]. The remaining sequences were realigned and used to generate maximum likelihood phylogenies in IQ-TREE v.1.6.9 [38]. Phylogenetic models were selected for each tree individually based on Bayesian Information Criteria using ModelFinder as implemented in IQ-TREE, and statistical support was assessed using 1000 ultrafast bootstrap pseudoreplicates [34, 54].

**Search for plastid localization signals**

To investigate the N-terminal extensions and thus intracellular location of proteins of interest, corresponding alignments were manually inspected for completeness of the sequences and for N-terminal extensions relative to prokaryotic or cytosolic homologs. Prediction of signal peptides as part of N-terminal bipartite leader sequences was performed with the Hidden Markov Model of SignalP3.0 using the default truncation setting of 70 residues [44]. To predict putative Nterminal transmembrane domains, TMHMM v.2.0 was used only on the first 100 amino acid residues of the transcript to improve prediction accuracy [45]. Putative plastid transit peptides were interpreted as 24-aa stretches downstream of the signal peptide, representing the minimum length for apicomplexan transit peptides still within the N-terminal extension and upstream of the estimated start of the mature protein, as described by Parsons et al. [55] Conserved domains and their coordinates in the mature protein region of candidate sequences were identified with the Pfam sequence search service on http://pfam.xfam.org/search/sequence, using the gathering threshold as a cut-off (Table S1).
Analysis of cellular metabolic pathways

We reconstructed the metabolic maps for our new transcriptomes, as well as representative species across the apicomplexan and chrompodellids, using Kyoto Encyclopedia of Genes and Genomes (KEGG) [46]. We first assigned KEGG ortholog identifiers (KO) to all proteomes using the web-based server, KAAS (KEGG Automatic Annotation Server) (https://www.genome.jp/kegg/kaas/), and where possible we used annotations already available within KEGG. The assigned KO numbers were used to identify complete metabolic pathways using the KEGG reconstruct module and module mapper. Complete metabolic pathways present in Piridium, Platyproteum or both but missing in other apicomplexans were further investigated. The identity of all proteins in these unique pathways were confirmed using BLASTp to ensure removal of contaminants and false positives (Table S2).

Ortholog identification and search for apicomplexan invasion/extracellular proteins

Orthofinder was used to infer orthologs within the apicomplexans, chrompodellids and Platyproteum, while Oxytricha, Tetrahymena, Symbiodinium and Perkinsus were used as a outgroup for the analyses [56]. Diamond searches were used to identify homologs between pairs of taxa [47]. The Orthofinder results were then analyzed using Dollo parsimony as implemented in the program Count to obtain estimates of gene gain and loss across different species [48]. Previously published sets of invasion (98 proteins from Plasmodium falciparum 3D7) and extracellular (722 proteins from diverse apicomplexans) proteins, as well as flagellar proteins, were then identified within orthogroups and their orthologs in the studied taxa were recorded (Table S3) [3, 4].

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical support for the phylogenomics tree was inferred using 500 bootstrap replicates using the LG+C40+I’4+F PMSF profiles, and 1000 bootstrap replicates using the LG+I’4+F model in RAxML [37, 50] (Figure 1). For the plastid based phylogenomic analyses, 500 non-parametric bootstrap replicates were used to assess support for the tree topology in RAxML (Figure S2) [37]. For the HMM search based phylogenies statistical support was assessed using 1000 ultrafast bootstrap pseudoreplicates in IQ-TREE [34].

DATA AND CODE AVAILABILITY

The genome and transcriptome sequencing reads are available in the NCBI Short Read Archive (SRA) NCBI: PRJNA539986. The Piridium sociabile plastid genome is available on GenBank: MK962129.
Supplemental Information

Multiple Independent Origins
of Apicomplexan-Like Parasites

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Figure S1. Myxozoan 18S SSU rRNA phylogenetic tree. Related to Figure 1.
The tree was inferred with Maximum Likelihood (TIM2+I+G4 IQ-TREE substitution model) and 1000 ultrafast bootstraps (UFB) support. The phylogeny includes a diversity of apicomplexans and chromodellids with dinoflagellate outgroups. All sequences were retrieved from GenBank and accession numbers are denoted beside the species name. Taxa in red represent 18S sequences that were retrieved from our sequenced transcriptomes. Black dots on nodes denote UFB > 96 and all other support values are written on the node.
Figure S2. Plastid phylogenomic tree. Related to Figure 2.
Maximum likelihood (ML) tree based on a concatenated alignment of 62 plastid genome encoded proteins. The ML tree was constructed in RAxML with the LG+GAMMA+F model of sequence evolution. Statistical support was inferred from 500 bootstrap replicates (BP) using the same model. Black dots denote 100% BP support.
Figure S3: Phylogenomic tree of the Apicomplexa and relatives (excluding Ascogregarina). Related to Figure 1. Maximum likelihood tree generated from an alignment comprising 198 genes and 58,116 sites under the C40+LG+Γ4+F substitution model with both non-parametric bootstraps (n = 500) shown. Black circles represent 100% bootstrap support and all other support values are indicated beside the node. Our newly sequenced transcriptomes are shown in bold red lettering.
**Supplemental References**
