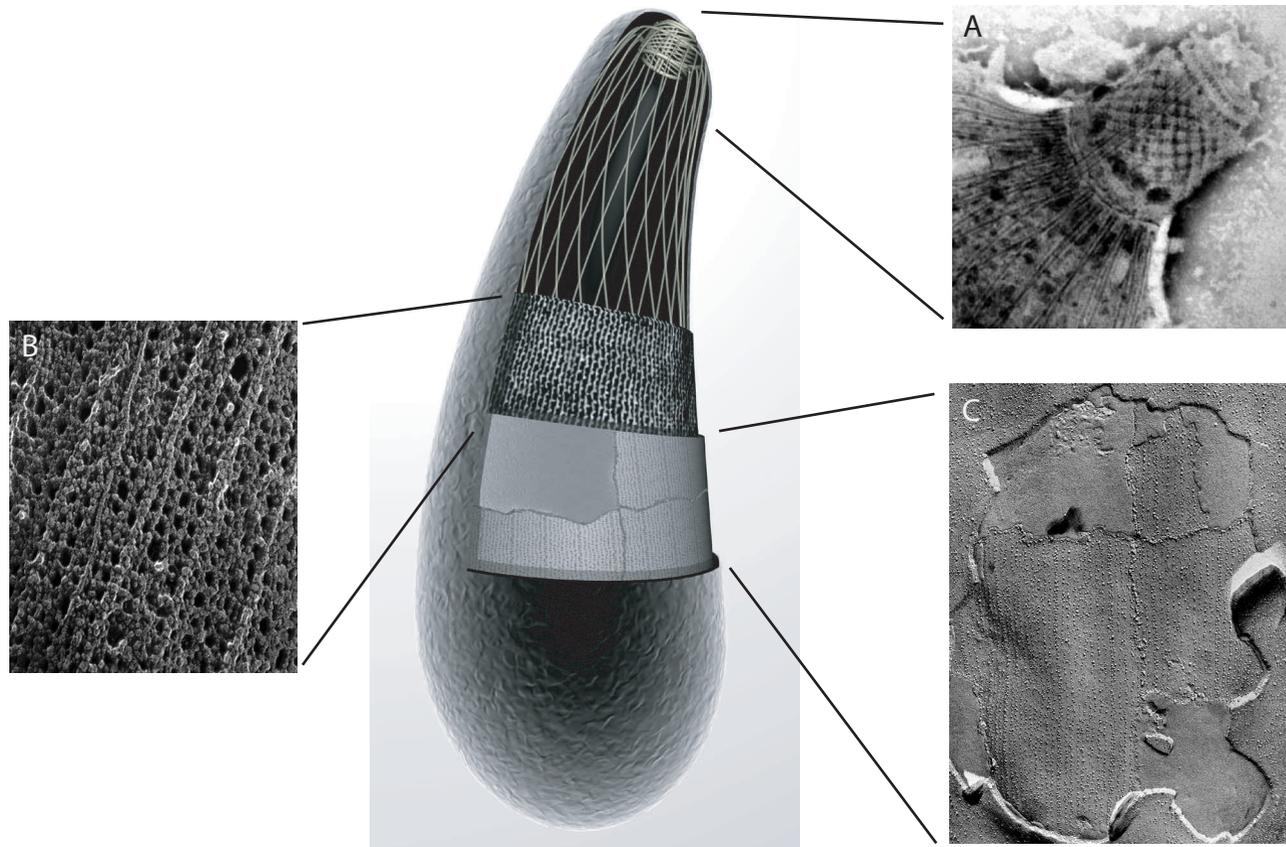


Cytoskeletal Organization

The phylum Apicomplexa (1, 2) is defined by its apical specialization and complex cytoskeleton. The cell cortex is comprised of several membranous layers and the underlying cytoskeleton. The arrangement of these layers is shown for *Toxoplasma gondii* as a representative example (Figure s1). Starting from the inner most layer, a rigid cell shape is imparted by a system of subpellicular microtubules that are collected at the anterior end by a unique organizing center called the conoid (3). Extending over the microtubules is a complex meshwork of filaments, comprised in part of the proteins IMC1 and IMC2 (4). The cell membrane consists of an outer plasma membrane underlain by a double, flattened membrane called the inner membrane complex (IMC). Freeze-fracture studies have revealed that the IMC contains numerous inner membrane particles that presumably represent proteins embedded within the membrane (5, 6).

Figure s1 Graphic depiction of the layers of *Toxoplasma gondii* seen in cut-away view. (A) The conoid is revealed by transmission EM after detergent extraction and negative staining as described previously (image used with permission (6)). (B) Encasing the microtubules is a meshwork of filaments observed by transmission EM after detergent extraction and platinum freeze-dried replica formation (image provided by Robin Roth and John Heuser, Washington University). The plasma membrane of the parasite is underlain by the inner membrane complex. (C) Freeze-etch replicas of this layer reveal that the IMC is comprised of two flattened cisternae that contain numerous inner membranous particles embedded in the membrane (image used with permission (6)). Graphic image was generated by Paul Warfel (University of Pennsylvania) with assistance of Naomi Morrissette (Washington University).

Figure 1

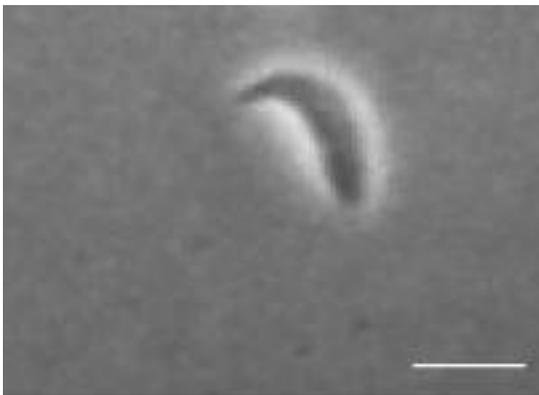


Gliding Motility

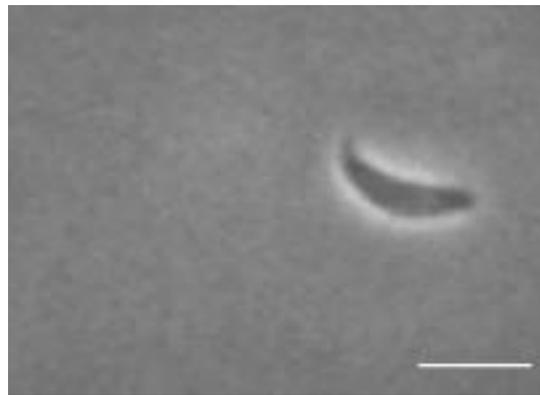
Apicomplexan parasites display a conserved behavior of substrate dependent motility termed “gliding” (7, 8). Apicomplexans are generally slender, crescent shaped cells that range in size from 4-9 microns long by 1-3 microns wide. The following video images depict gliding motility as it occurs *in vitro* by a variety of apicomplexans.

Toxoplasma

Movies depicting gliding motility by *Toxoplasma gondii* tachyzoites were recorded by Sebastian Håkansson (Washington University) as described previously (9). Videos are projected at twice normal speed. During circular gliding (Movie s1) the parasite traces a circular pattern, moving counterclockwise (when viewed from above) while lying on its right side (the convex side being defined as dorsal). Helical (Movie s2) gliding occurs in a clockwise manner and involves rotation about the long axis of the cell. During helical gliding the parasite traces out an arc along the dorsal surface of the cell. Helical gliding involves two different phases as described previously (9). During the first 180° rotation, the parasite moves forward one body length. It then flips from the right side to the left side in a rapid motion that does not result in net forward movement. The successive rotational movements trace out a roughly linear pattern across the substrate. Importantly, only helical gliding leads to cell entry (9).



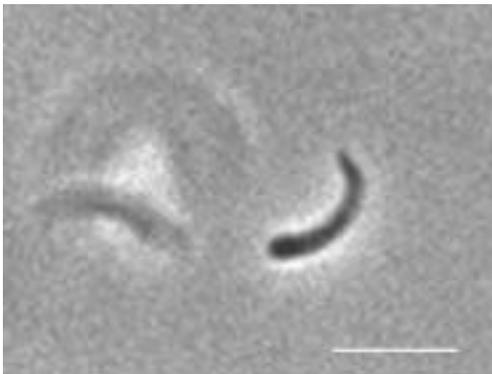
Movie still s1 Circular gliding by *T. gondii* tachyzoites. Scale bar = 5 microns



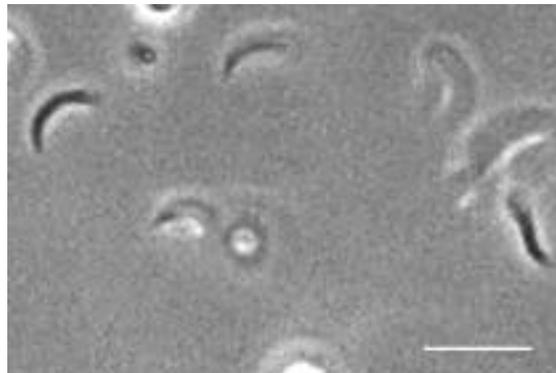
Movie still s2 Helical gliding by *T. gondii* tachyzoites. Scale bar = 5 microns

Cryptosporidium

Movies depicting gliding motility by *Cryptosporidium parvum* sporozoites were recorded by Dawn Wetzel (Washington University) with assistance from Joanne Schmidt (University of Illinois) as described previously (10). Movies are projected at 1X original speed. Gliding motility by *Cryptosporidium* is analogous to that described above in *Toxoplasma*. The parasite moves in either circular (Movie s3) or helical patterns (Movie s4). Helical gliding traces a more undulating path than with *Toxoplasma*. This difference may reflect the fact that *Cryptosporidium* sporozoites are more slender. Moreover, sporozoite gliding is significantly faster than by *Toxoplasma* tachyzoites. *Cryptosporidium* gliding results in the deposit of trails on the substrate (11), similar to *Toxoplasma* (9).



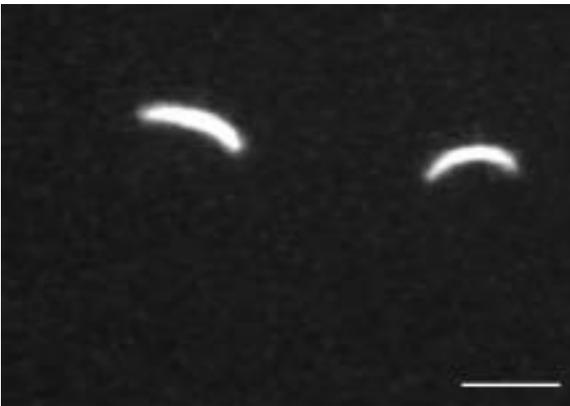
Movie still s3 Circular gliding by *C. parvum* sporozoites. Scale bar = 5 microns.



Movie still s4 Helical gliding by *C. parvum* sporozoites. Scale bar = 5 microns.

Plasmodium

Plasmodium sporozoites also display gliding motility on solid substrates. Gliding is characterized by circular patterns (Movie s5) that result in deposits of trails on the substrate (12). Plasmodium sporozoites also undergo undulating helical gliding as described previously (13) and depicted in Movie s6. During helical gliding, the parasite adopts a more accentuated bend in the middle of the cell body compared to Toxoplasma, and this pattern is also seen in *Cyrtosporidium* (Movie 4). Like in the case of *Toxoplasma*, helical gliding proceeds cell invasion by malaria sporozoites (Movie s7).



Movie still s5 Circular gliding of *P. berghei* sporozoites. Provided by J. Dvorak (NIH). Elapsed time shown in movie. Scale bar = 10 microns.



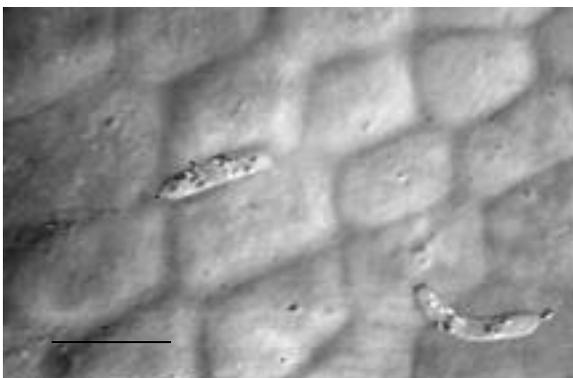
Movie still s6 Helical gliding of *P. berghei* sporozoites. Provided by J. Vanderberg (NYU). Elapsed time shown. Scale bar = 10 microns.



Movie still s7 Helical gliding by *P. berghei* sporozoites proceeds invasion of macrophage *in vitro*. Provided by J. Vanderberg (NYU). Elapsed time shown. Scale bar = 10 microns.

Supplemental Online Materials

During development in the insect, the ookinate form also displays gliding motility (Movie s8). Ookinates develop from the zygote after fertilization occurs in the mosquito midgut lumen following a blood meal. The ookinate penetrates the midgut epithelium, crossing this barrier to develop into an oocyst on the basolateral surface. Passage of the parasite across the epithelium inflicts damage on the epithelium and leads to cell death (14, 15).



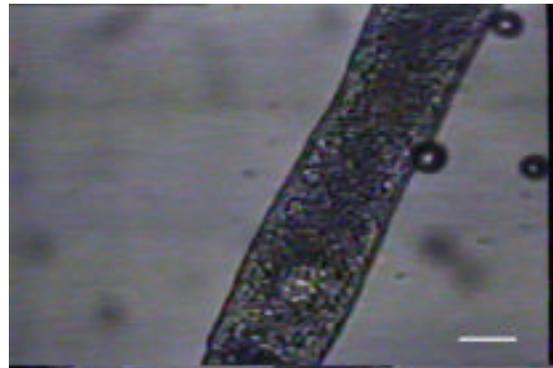
Movie still s8 Gliding motility of *P. berghei* ookinates on insect midgut epithelial cells. Gliding occurs across the surface of the cells, invasion occurs along the lateral edge from the apical surface. Provided by J. Dvorak (NIH). Scale bar = 10 microns.

Gregarina

Gregarines are enteric parasites of invertebrates. They are grouped in the Apicomplexa based on their life cycle features and conserved SSU sequences, which reveal they are most closely aligned with *Cryptosporidium* (16, 17). The images shown here represent *Gregarina polymorpha* that were recovered from the flour beetle (*Tenebrio molitor*). Gliding on the substrate results in gradual forward movement (left to right in Movie s9). A similar mechanism results in translocation of 10 micron beads along the surface, resulting in accumulation at the posterior end of the cell (top in Movie s10). Gregarina movies are projected at 1X normal speed. Motility of Gregarines is further described in (7, 8, 18).



Movie still s9 Gliding motility by *Gregarina polymorpha*. Provided by C. King (University College, London). Scale bar = 20 microns.



Movie still s10 Translocation of beads among the surface of Gregarina. Provided by C. King (University College, London). Scale bar = 20 microns.

References

1. N. S. Morrissette, L. D. Sibley, *Microbiol. Mol. Biol. Rev.* **66**, 21 (2002).
2. B. Nichols, M. Chiappino, *J. Protozool.* **34**, 217 (1987).
3. K. Hu, D. S. Roos, J. M. Murray, *J. Cell Biol.* **156**, 1039 (2002).
4. T. Mann, C. Beckers, *Molec. Biochem. Parasitol.* **115**, 257 (2001).
5. E. Ferreira, J. F. Dubremetz, *J. Protozool.* **29**, 304 (1982).
6. N. S. Morrissette, J. M. Murray, D. S. Roos, *J. Cell Sci.* **110**, 35 (1997).
7. C. A. King, *Parasitol. Today* **11**, 315 (1988).
8. T. M. Preston, C. A. King, *Acta Protozoologica* **35**, 3 (1996).
9. S. Håkansson, H. Morisaki, J. E. Heuser, L. D. Sibley, *Molec. Biol. Cell* **10**, 3539 (1999).
10. D. M. Wetzel, S. Håkansson, K. Hu, D. S. Roos, L. D. Sibley, *Mol. Biol. Cell* **14**, 396 (2003).
11. M. J. Arrowood, C. R. Sterling, M. C. Healey, *J. Parasitol.* **77**, 315 (1991).
12. M. J. Stewart, J. P. Vanderberg, *J. Protozool.* **35**, 389 (1988).
13. J.P. Vanderberg, S. Chew, M.J. Stewart, *J. Protozool.* **37**, 528 (1990)
14. Y. S. Han, J. K. Thompson, F. C. Kafatos, C. Barillas-Mury, *EMBO J.* **19**, 6030 (2000).
15. H. Zieler, J. A. Dvorakk, *Proc. Natl. Acad. Sci. (USA)* **97**, 11516 (2000).
16. J. R. Barta, *Vet. Parasitol.* **101**, 175 (2001).
17. R. A. Carreno, D. S. Martin, J. R. Barta, *Parasitol. Res.* **85**, 899 (1999).
19. C. A. King, *Cell Biol. Intl. Rep.* **5**, 297 (1981).