Life cycle stages, specific organelles and invasion mechanisms of *Eimeria* species

Alana Burrell[1,2], Fiona M. Tomley[1], Sue Vaughan[2], Virginia Marugan-Hernandez[1*]

[1] The Royal Veterinary College, University of London, Hawkshead Lane, North Mymms, AL9 7TA, UK

[2] Department of Biological and Medical Sciences, Oxford Brookes University, Gipsy Lane, Oxford, OX3 0BP, UK

* Corresponding author: Virginia Marugan-Hernandez, The Royal Veterinary College, University of London, Hawkshead Lane, North Mymms, AL9 7TA, UK, Tel. +44(0)1707669445, E-mail vhernandez@rvc.ac.uk, ORCID https://orcid.org/0000-0003-1512-4682

11 SUMMARY

12 Apicomplexans, including species of *Eimeria,* pose a real threat to the health and wellbeing of animals
13 and humans. *Eimeria* parasites do not infect humans but cause an important economic impact in
14 livestock, in particular to the poultry industry. Despite its high prevalence and financial costs, little is
15 known about the cell biology of these 'cosmopolitan' parasites found all over the world. In this review
16 we discuss different aspects of the life cycle and stages of *Eimeria* species, focusing on cellular
17 structures and organelles typical of the coccidian family as well as genus-specific features,
18 complementing some 'unknowns' with what is described in the closely related coccidian *Toxoplasma*
19 *gondii*.

20

21 Key words: Coccidia*, Eimeria*, cell biology, life cycle stages, secretory organelles, invasion

INTRODUCTION

Eimeria species are protozoan parasites belonging to the Coccidiasina (Coccidia) (Fig. 1), a group of obligate intracellular parasites of great human and veterinary importance (Shirley *et al.*, 2005). All members of the Coccidia replicate within the intestines of a definitive host progressing through sequential rounds of asexual (schizogony) and sexual (gametogony) reproduction, culminating in the production of oocysts that are shed into the environment with the faeces (Kemp *et al.*, 2013). Coccidians of the family *Eimeriidae*, such as species of *Eimeria* and *Cystoisospora*, are monoxenes meaning that their development is restricted to a single host where they replicate rapidly to reach high numbers in the intestine causing acute enteritis of varying severity. This is in contrast to coccidians of the family *Sarcocystidae*, such as *Toxoplasma gondii* and *Neospora caninum*, which are heteroxenes that complete their whole cycle within the intestines of a definitive host and can also undergo asexual replication within a variety of intermediate host species. Here, infection progresses through an acute, rapid-replication phase into a chronic phase where parasites persist as cysts, most commonly in brain and muscle tissues (Wohlfert *et al.*, 2017). Heteroxene Coccidia may also form tissue cysts in the definitive host where they remain dormant throughout the life of the host, but can reactivate into the acute phase and can cause a range of diseases including encephalitis and abortion.

In contrast to the very broad host range of *T. gondii* (Cowper et al., 2012) species of *Eimeria* are highly host adapted and are generally capable of parasitising a specific intestinal location in a single host (Augustine, 2001; Cowper *et al.*, 2012; Yun *et al.*, 2000a). In Dobell's paper entitled 'The Discovery of the Coccidia' (Dobell, 1922) he concludes that it was Leeuwenhoek who first described an *Eimeria* specie some 200 years before the conception of the genus by Schneider in 1875 (Schneider, 1875): in one of his letters in 1674, Leeuwenhoek describes numerous microscopic globules in the bile of rabbits which Dobell believes must have been oocysts of *Eimeria stiedae*. In 1879, Leuckart founded the class Sporozoa, grouping together the Coccidia and similar organisms which are encased within a protective 'spore' covering (Leuckart, 1879). In the early 20th century, light microscopists were able to describe

in detail the life cycles of *Eimeria* spp. and reveal their intimate connection with the tissues of the host (Fantham, 1910). By the late 90's many electron microscopy studies had been performed, characterising organelle and cytoskeletal make-up of *Eimeria* spp. (Ryley, 1969). Electron microscopy was also instrumental to understanding how apicomplexans enter and reside within host cells (Aikawa *et al.*, 1978; Scholtyseck, 1965; Sheffield & Hammond, 1966).

Eimeria is a large genus, with over 1800 species identified to date (Duszynski, 2001). Despite exquisite host specificity of individual species, the genus as a whole has a hugely diverse host range and affects members of all vertebrate classes (Duszynski, 2001). Interestingly, one host not affected by *Eimeria* is *Homo sapiens* (Relman *et al.*, 1996). Humans are however specifically infected by *Cyclospora cayetanensis,* a parasite which is highly similar to *Eimeria* species in terms of both genetics and pathogenesis (Liu *et al.*, 2016) and is currently considered as the 'human *Eimeria*'. As the replication stages of schizogony and gametogony occur within host cells, infection with *Eimeria* species results in cellular destruction and pathology to the susceptible host. The usual site of this replication is within epithelial cells lining the intestinal tract. This can lead to clinical symptoms of gastrointestinal dysfunction such as diarrhoea, dehydration and failure to gain weight (Yun *et al.*, 2000b). The diseases caused by *Eimeria* spp. are commonly known as 'coccidiosis' or in some cases 'eimeriosis'.

The following sections predominantly refer to research involving species of *Eimeria*; where data involving *T. gondii* is used, for example where equivalent studies in *Eimeria* species are missing, this is stated within the text.

CHICKEN COCCIDIOSIS

The most economically important disease caused by *Eimeria* species, is coccidiosis in chickens. The estimated global cost of this disease, as stated in a review by Peek and Landman, is more than two billion US dollars per year through production losses and costs associated with treatment and prevention measures (Peek & Landman, 2011). There are seven recognised species of *Eimeria*

affecting chickens: *E. acervulina, E. brunetti, E.maxima, E. mitis, E. necatrix, E. praecox and E. tenella* (Shirley et al., 2007; Shirley et al., 2005). Previously described species *E. mivati* and *E. hagani* (Edgar & Seibold, 1964) (Levine, 1938) are regarded as *nomina dubia* by the vast majority of coccidiologists and to our knowledge there are no isolates of these available upon which definitive molecular tests could be applied. There are however a number of hitherto unclassified isolates, referred to as operational taxonomic units (OTU) X, Y and Z that are widely distributed throughout the world and may represent novel cryptic species (Clark *et al.*, 2016; Jatau *et al.*, 2016). Advances in this area for a final classification would be necessary for accurate control strategies for these isolates.

Each of the seven recognised species has a set of distinct characteristics in terms of prevalence, pathogenicity, site of infection in the intestine and oocyst morphology (Table 1). *Eimeria tenella* specifically targets the paired caeca, often resulting in fairly extensive haemorrhage; the lesions are primarily caused by second generation schizonts that develop deep to the intestinal epithelium within the lamina propria (Fernando *et al.*, 1983). Infection with *E. maxima* is likely to cause a thickening of the intestinal lining, with mucoid to bloody exudate, and *E. acervulina* is described as causing 'ladder-like' white bands across the mucosa. *Eimeria brunetti* and *E. necatrix* are also capable of causing severe pathology, however they are less commonly encountered than *E. acervulina*, *E. maxima* and *E. tenella* (Trees, 2001).

THE LIFE CYCLE OF *EIMERIA* SPECIES

All *Eimeria* species infecting chickens exhibit a similar life cycle (Lillehoj & Trout, 1993) (Fig. 2). Unsporulated oocysts are released in the faeces of an infected animal and persist in the environment for long periods. When exposed to air, moisture and warmth, oocysts go through a developmental process called sporulation (Shirley *et al.*, 2005) (Fig. 2.1). If ingested by a chicken, the sporulated oocyst will release sporocysts (Fig. 2.2). As these pass into the small intestine, enzymatic digestion releases the sporozoites, which migrate to their preferred site of development to initiate cellular

invasion (Jeurissen *et al.*, 1996) (Fig. 2.3). The developmental cycle in the host cell begins with two to three rounds of asexual replication known as schizogony. This process involves multiple nuclear divisions to produce a large multinuclear cell called schizont, from which merozoites are formed (Fig. 2.4 and 2.5). After several generations of merozoite production, parasites development proceeds with a single round of sexual replication known as gametogony (Fig. 2.6), forming the dimorphic stages of macrogamete and microgamete. Ultimately, macrogamete/microgamete fertilisation occurs to form a zygote (Ferguson *et al.*, 2003). The zygote will then develop into an oocyst, which, after release in the faeces, matures into an infective sporulated oocyst (Jeurissen *et al.*, 1996; Shirley *et al.*, 2005) (Fig. 2.7).

The infective oocyst

The oocyst, which has important roles in both parasite development and disease propagation (Lillehoj & Trout, 1993), can persist in the environment for long periods, even in the presence of disinfectants (Peek & Landman, 2011). This is largely due to protection afforded by the oocyst wall, measuring around 100nm in thickness and formed from two opposed but easily separated layers and composed of a mixture of lipids and glycoproteins (Belli *et al.*, 2006; Mai *et al.*, 2009; Stotish *et al.*, 1978). The final stage of oocyst development (sporulation) occurs outside of the host and involves a single meiotic division followed by a round of mitosis, resulting in eight infective parasite stages (sporozoites) arranged as pairs inside four individual casings (sporocysts) within each oocyst (Canning & Anwar, 1968) (Figs. 2.1 and 3). As well as containing two sporozoites, each sporocyst contains a micropyle at the apex and a lipid-rich residual body which remains within the sporocyst following sporozoite excystation. The stieda and sub-stieda bodies are found at the anterior pole of the sporocyst, acting as a barrier to sporozoite release until their degradation in the presence of trypsin (Roberts *et al.*, 1970b).

The invasive sporozoite

Most knowledge on the sporozoite comes from *E. tenella*, the most extensively studied of the avian *Eimeria* species due to its amenability for sporozoite invasion and schizont development *in vitro,* as well as its potential for genetic manipulation. The *E. tenella* sporozoite is a distinctly polarized cell, of sickle shape and around 10µm in length (Figs. 4.A and 5.B). Sporozoites contain many classical features and organelles of typical eukaryotic cells such as the nucleus, mitochondrion, endoplasmic reticulum and Golgi apparatus (Fig. 4.D,E) . They also contain several features specific to the phylum (such as the conoid, apicoplast, micronemes and rhoptries, Fig. 4.B,C,G and 5.A), and even structures which appear to be unique to this genus (refractile bodies, Fig. 4.A and 5.B). The *E. tenella* sporozoite nucleus is situated in roughly the centre of the parasite (Fig. 4.A), contains a nucleoplasm of fine granular consistency and is surrounded by a pore-containing double membrane (Pacheco *et al.*, 1975; Strout & Scholtyseck, 1970). Adjacent to the nucleus is the Golgi apparatus (Fig. 4.D), which contains material described as 'small vesicles in a finely granular matrix' (Vetterling *et al.*, 1973). The mitochondrion of a *T. gondii* tachyzoite, reconstructed from serial electron microscopy data, was found to be a single elongated structure distributed throughout the cell (Melo *et al.*, 2000) (Fig. 5.A).

Several early investigations into the structure and composition of the *Eimeria* cell surface revealed the presence of an inner membrane complex (IMC) composed of a meshwork of flattened sacs found directly beneath the outer plasma membrane (Fig. 4.F) (Dubremetz, 1975; Dubremetz & Torpier, 1978). Building on the work by Dubremetz *et. al*., (1975, 1978), studies of *T. gondii* tachyzoites have shown that the IMC is connected to the subpellicular microtubules via a network of intermediate filaments and alveolin (Morrissette & Sibley, 2002). They have an important role in maintaining cell rigidity, critical for assembly and stabilisation of the actin-myosin motor that powers parasite gliding motility (Sibley, 2010), a point of attachment for organelles (Kudryashev *et al.*, 2010) and apparently originates from the Golgi apparatus (Francia & Striepen, 2014).

Beneath the pellicle there is a set of longitudinally running microtubules. One of the first depictions of this cytoskeletal organisation was achieved in *E. acervulina* through 'critical point drying' (a method

144 of dehydrating a biological sample without distorting morphology), allowing the isolation and electron
145 microscopy examination of the outer cytoskeletal microtubules (Russell & Sinden, 1982). More
146 recently, several reviews have focused on the architecture and role in invasion of the cytoskeleton in
147 coccidian parasites (Frenal & Soldati-Favre, 2009; Morrissette & Sibley, 2002; Sibley, 2010). The 24
148 subpellicular microtubules of *Eimeria* species are arranged as an evenly spaced spiral and extend to
149 roughly half of the parasite length, acting as a support scaffold to maintain cell shape and rigidity
150 (Morrissette & Sibley, 2002; Russell & Sinden, 1982; Vetterling *et al.*, 1973). The apical ends of these
151 microtubules are fixed in a ring around the conoid, forming the apical polar ring (APR), however it is
152 not clear whether their posterior ends are fixed or free (Russell & Sinden, 1982; Vetterling *et al.*, 1973).

153 *i. Refractile bodies, amylopectin granules and acidocalcisomes*

154 In light and electron micrographs of *E. tenella* sporozoites, the most striking structures are the
155 refractile bodies (RB) (Fig. 4.A and 5.B). On transmission electron micrographs these appear as
156 spherical or ovoid structures with homogeneous electron dense content and no obvious limiting
157 membrane. Usually a spherical RB is found anterior to the nucleus (around 1-2µm in diameter) and an
158 ovoid RB is found posterior to the nucleus (around 5µm in length). Despite their striking appearance,
159 the functions of RBs remain unknown; the most commonly hypothesised roles are protein storage and
160 metabolism (de Venevelles *et al.*, 2006). Work by Fayer described an interesting dynamic, whereby
161 after host cell invasion the anterior and posterior RB merge into a single RB that localises centrally in
162 the schizont (Fayer, 1969). Twenty years later a monoclonal antibody (1209-C2) specific for RBs (and
163 able to bind related *E. tenella* proteins) was used to show that the merged RB separates into globules
164 that diffuse through the schizont and re-concentrate as a refractile 'dot' within each first-generation
165 merozoite (Danforth & Augustine, 1989). The protein target of the 1209-C2 was identified
166 independently by two research groups seeking to validate vaccine antigens for control of coccidiosis
167 (Crane *et al.*, 1991; Miller *et al.*, 1989). This RB protein, termed variously GX3262, 'B' antigen and SO7,
168 contain large numbers of repetitive amino acid tracts (Liberator *et al.*, 1989; Reid *et al.*, 2014) and

induce partial protection against challenge with up to four different species of *Eimeria* (Bhogal *et al.*, 1989; Karkhanis *et al.*, 1991). SO7 (most common name) has been used as a candidate antigen in a variety of vaccinology studies (Song *et al.*, 2015; Yang *et al.*, 2017) although the mechanism of immune protection, and the biological function of this family of RB proteins, that are unique to the genus, remain unknown. A few additional proteins have been localised to the RBs including an aspartyl protease (Laurent *et al.*, 1993), a putative nucleotide transhydrogenase (Vermeulen *et al.*, 1993) and some other up-represented in a RB enriched proteome such as a lactate dehydrogenase, a carbonyl reductase, a subtilisin 2 protease and a haloacid dehalogenase-like hydrolase (de Venevelles *et al.*, 2006).

Surrounding the larger (posterior) RB and dispersed throughout the posterior two thirds of the parasite, are several tens of amylopectin granules (Pacheco *et al.*, 1975; Vetterling *et al.*, 1973) (Fig. 5.B). These are smaller than the RBs and easily identified on transmission electron micrographs (TEM), appearing electron dense or lucent dependent on the staining technique (Fernando & Remmler, 1974; Ryley *et al.*, 1969; Strout & Scholtyseck, 1970). Amylopectin is an important carbohydrate source for *Eimeria*, being present in the oocyst, sporozoite and merozoite stages, as well as in the residual body of the schizont (Coombs *et al.*, 1997; Ryley *et al.*, 1969). In *T. gondii,* amylopectin granules are much more numerous in bradyzoites (the latent tissue cyst stage) than tachyzoites (the rapid proliferation stage) suggesting they could be used to fuel transition between these two stages (Guerardel *et al.*, 2005). *Eimeria* species contain another group of organelles that appear by TEM as membrane bound spherical structures roughly half a micron in diameter and filled to varying extents with highly electron dense material. In *T. gondii* analogous structures are classified as acidocalcisomes: acidic organelles containing high concentrations of various physiologically important ions (Miranda *et al.*, 2008; Soares Medeiros *et al.*, 2011) (Fig. 5.A).

ii. Conoid

193 The conoid is a movable cone-shaped structure which sits within the apical polar ring (APR), from
194 which the subpellicular microtubules emanate (Ryley, 1969; Vetterling *et al.*, 1973) (Fig. 5). In *T. gondii,*
195 the conoid was found to be composed of a novel tubulin polymer, arranged as in a tight spiral of
196 'microtubule-like' fibres (Hu *et al.*, 2002). Studies on the molecular composition of the *T. gondii* conoid
197 revealed the presence of several additional novel proteins (Hu *et al.*, 2006), including three
198 calmodulin-like proteins involved in motility, invasion and egress, but not required for conoid
199 extrusion or microneme/rhoptry secretion (Long *et al.*, 2017b), as well as an essential protein (CPH1)
200 involved in conoid stability (Long *et al.*, 2017a). In several species of *Eimeria,* the conoid has been
201 observed in both extended and retracted states (Roberts & Hammond, 1970). However, in electron
202 micrographs depicting extracellular, invading or intracellular *Eimeria* sporozoites, the conoid is rarely
203 seen in the extended position (Jensen, 1975; Roberts *et al.*, 1970a; Roberts *et al.*, 1971; Vetterling *et*
204 *al.*, 1973). In *T. gondii* the conoid has been shown to rotate, tilt, extend and retract (Bommer *et al.*,
205 1969), often seen in the extended state immediately prior to invasion (Carruthers & Boothroyd, 2007;
206 Chiappino *et al.*, 1984). This range of movement has led to the hypothesis that the conoid has a
207 mechanical role in invasion (Morrissette & Sibley, 2002).

208 *iii. Micronemes and rhoptries*

209 Micronemes are small (20nm by 50nm) rod-shaped organelles located at the anterior end of the zoite
210 (Fig. 4.B). Their protein content is well defined in several coccidians, including *E. tenella* and *T. gondii.*
211 There are genus-specific differences in the precise portfolio of MICs but overall these are generally
212 conserved, comprising a mix of soluble and membrane-spanning proteins, many with domains
213 orthologous to known adhesins (reviewed by Carruthers & Tomley, 2008; Cowper *et al.*, 2012; Tomley
214 & Soldati, 2001). Surface-expressed MICs are able to bind host ligands (essential for gliding motility,
215 attachment and invasion of host cells) and also bind parasite-derived RON ligands to form the moving
216 junction (discussed later).

Rhoptries are club-shaped organelles (up to around 2,000nm in length) often seen with the narrow neck portion extending longitudinally into the conoidal channel (Fig. 5A, Fig. 5B). It is not clear precisely how many rhoptries each sporozoite contains, although up to eight have been seen for *E. tenella* (Pacheco *et al.*, 1975). Rhoptry proteins are housed in two compartments: RONs are stored in the rhoptry necks and ROPs come from the bulbous part of the organelle (Besteiro *et al.*, 2011).

iv. Biogenesis of the microneme and rhoptry

The apical organelles are part of the parasite endomembrane system and in *E. tenella* are generated *de novo* late in the formation of sporozoites (Ryan *et al.*, 2000) and merozoites (Brown *et al.*, 2000; Tomley & Soldati, 2001). From studies in *T. gondii* and *Plasmodium* species it is well established that apicomplexans have re-purposed the exocytic and endocytic pathways of higher eukaryotes into a partially 'merged' vesicle and protein trafficking system in order to generate their specialized regulatory secretory organelles (reviewed by (McGovern & Carruthers, 2016; Tomavo *et al.*, 2013). Microneme (MIC) and rhoptry (ROP/RON) proteins enter the early exocytic pathway at the ER and traffic to the Golgi and trans-Golgi network (TGN), from where they are sorted to novel endosome-like compartments (ELC) bearing early (Rab5) or late (Rab7) endosome markers. In *T. gondii*, over-expression of Rab5A/5C causes defective trafficking of some (but not all) MIC and all ROP/RON proteins presumably by saturating receptors and dysregulating vesicle transport (Kremer *et al.*, 2013).

Several additional molecules are essential for TGN to ELC trafficking of MIC and ROP/RON proteins in *T. gondii* and as gene orthologues are present in *Eimeria* species (www.toxodb.org) it is likely that this trafficking pathway is conserved across the Coccidia. These molecules include vacuolar sorting protein 9, an activator of Rab5 (Sakura *et al.*, 2016); syntaxin 6 (Stx6), a SNARE found mainly in the TGN (Jackson *et al.*, 2013); dynamin-related protein B (DrpB) and clathrin, both found in the TGN and ELC (Breinich *et al.*, 2009; Pieperhoff *et al.*, 2013); the clathrin adaptor complex AP1 (Venugopal *et al.*, 2017); the sortilin-like receptor TgSORTLR (Sloves *et al.*, 2012); and several components of CORVET

(class C core vacuole/endosome tethering) and HOPS (homotypic fusion and vacuolar protein sorting) complexes associated with early and late endosomes respectively (Morlon-Guyot *et al.*, 2018; Morlon-Guyot *et al.*, 2015). TgSORTLR loads MIC and ROP/RON proteins at the TGN and escorts them to the ELC (Sangare *et al.*, 2016; Sloves *et al.*, 2012). In higher eukaryotes, sortilin is a transmembrane endosomal receptor with a major role in anterograde transport of lysosomal enzymes from TGN to endosomes (Bonifacino & Rojas, 2006), and in retrograde trafficking where it binds retromer, an evolutionarily conserved protein complex that selects and recycles proteins from endosomes to the TGN or the plasma membrane (Pan *et al.*, 2017). Recent characterization of retromer interactomes in *T. gondii* confirms that retromer-dependent retrograde transport is essential for apical organelle biogenesis, probably because recycling of TgSORTLR to the TGN is needed to maintain anterograde trafficking of MIC and ROP/RON proteins to the ELC (Sangare *et al.*, 2016).

Because not all MIC proteins depend on Rab5A/5C trafficking, it is suggested there is more than one trafficking pathway leading to either distinct populations of micronemes containing different MICs, or a single population having different sub-compartments (Kremer *et al.*, 2013). More evidence for dual trafficking emerged recently with the observation that vacuolar protein sorting 8 (TgVps8, a CORVET component), whilst essential for organelle biogenesis, completely blocks trafficking of most MICs but only partially affects that of TgAMA1, TgMIC2 and TgMIC6 (Morlon-Guyot *et al.*, 2018). No work on MIC trafficking has been carried out in *Eimeria* species, but lack of co-localisation of MIC proteins has been observed, for example between EtMIC3/EtAMA1, and between EtMIC3/EtMIC5 in sporozoites (Lai *et al.*, 2011), and between EtMIC2/EtAMA2 in second generation merozoites (McGovern *et al.*, 2018; Pastor-Fernandez *et al.*, 2018). These studies used non-quantitative imaging and whilst strongly suggestive that zoites contain distinct populations of micronemes, it cannot be ruled out that they reflect differences in timing and levels of individual MIC protein expression.

By merging and adapting exocytosis and endocytosis, apicomplexans have conserved an endomembrane system that is much reduced compared to higher eukaryotes, but efficient to perform

the protein trafficking, targeting, processing and re-cycling needed for their obligate intracellular lifestyles. How MIC and ROP/RON proteins are differentially sorted and trafficked beyond the ELC to their target organelles, and how the endomembrane system efficiently segregates exocytosis from endocytosis are major questions. In *T. gondii* divergence between MIC and RON/ROP proteins at the ELC has been noted, with immature MICs (TgM2AP and TgMIC5) located in ELC that also contain endocytosed host protein whilst an immature RON (TgRON4) is in ELC lacking ingested host protein (McGovern *et al.*, 2018). Thus endocytosis appears to intersect directly with MIC exocytosis and there must be a mechanism for directing MICs from ELC to micronemes and away from ingested host proteins that are *en route* to the parasite lysosome-like vacuole (VAC, Dou *et al.*, 2014). In contrast, trafficking of RON/ROP proteins appears to avoid contact with endocytosed host protein, proceeding via an immature pro-rhoptry compartment (McGovern *et al.*, 2018). Nevertheless, successful rhoptry biogenesis requires the endosomal CORVET protein TgVps9, and a novel parasite BEACH domain-containing protein that is also essential for VAC formation (Morlon-Guyot *et al.*, 2018).

An essential step in late exocytosis of MIC and RON/ROP proteins is proteolytic processing (Nishi *et al.*, 2008) that most likely occurs within, or during exit from the ELC (McGovern *et al.*, 2018) mediated by proteinases that include aspartyl protease 3 (Dogga *et al.*, 2017). Failure to undergo proteolytic maturation results in impaired organelle formation. Interestingly, apicomplexans possess a family of four phylogenetically related transporters belonging to the major facilitator superfamily (MFS) and termed transporter family protein 1 to 4 (TFP1-4) (Besteiro *et al.*, 2011; Hammoudi *et al.*, 2018). In *T. gondii* TFP1 localizes to micronemes and ELC and is critical for condensation of microneme content, presumably by allowing the transport of molecules that are essential this process such as maturases, or chaperones. Knock down of TFP1 impairs microneme formation and completely blocks MIC exocytosis; TFP2 and 3 localise to the rhoptries and knock down of TFP2 results in elongated rhoptries, again suggesting that defects in condensation/compaction have an impact on the late stages of organelle biogenesis (Hammoudi *et al.*, 2018).

291 *Zoite invasion of host cells*

292 A widely researched aspect of coccidian biology is the mechanism by which zoites (sporozoites,
293 merozoites, tachyzoites and bradyzoites) invade the host cell to occupy a unique intracellular niche,
294 the PV. Much of the molecular detail of this process has been described in the *T. gondii* tachyzoite, a
295 model cell for coccidian parasites. Considering the biological differences between tachyzoites and
296 sporozoites (tachyzoites are formed when a sporozoite or bradyzoite stage converts within the cells
297 of a host, rapidly replicates and disseminates throughout the host, then converts back into
298 bradyzoites) (Dubey *et al.*, 1998), we must be careful when inferring knowledge from *T. gondii*
299 tachyzoites to *Eimeria* where there is no such stage-conversion (to tachyzoite or bradyzoite) or
300 persistent infection. However, at the genomic and cellular levels much of the complex invasion
301 machinery used by the coccidia is conserved so it is useful to supplement rather sparse data on *Eimeria*
302 species with knowledge from *T. gondii*.

303 *i. The process of invasion*

304 Following initial contact with a host cell *in vitro*, the *E. tenella* sporozoite glides across the cell surface
305 in a helical motion, possibly in a search of an appropriate location to invade (Entzeroth *et al.*, 1989;
306 Russell & Sinden, 1981). Before invasion, the sporozoite re-orientates itself so that the apical tip, at
307 which the conoid is located, makes contact with the host cell plasma membrane forming a 'moving
308 junction' (MJ) between parasite and host cell membranes through which the parasite propels itself to
309 enter the newly forming intracellular vacuole. As the parasite pushes itself into the cell, it causes an
310 invagination of the membrane. The MJ remains fixed at the point of attachment to the host cell but is
311 translocated backwards over the surface of the invading parasite from apex to posterior. This
312 invagination continues until the whole parasite length has passed through the MJ, at which point the
313 host membrane pinches together behind the parasite posterior enclosing the parasite within a PV.
314 This process has been well documented in several species of *Eimeria* as well as *T. gondii* and other

apicomplexans (Beyer *et al.*, 2002; Entzeroth *et al.*, 1998; Suss-Toby *et al.*, 1996). According to this model, the parasite does not enter the host cell cytoplasm, although there is evidence that some of the rhoptry content enters in the form of e-vacuoles (Hakansson *et al.*, 2001), a process that may be critical for the early release of rhoptry neck proteins (RONs) and formation of the MJ (Besteiro *et al.*, 2011). In addition to stabilising the site of invasion, the MJ has a role as a molecular sieve, removing non-GPI-anchored host membrane proteins from the newly formed PV membrane, including the key immune signalling/effector molecules MHC class I, MHC class II and FcR (Mordue & Sibley, 1997). The PV of *T. gondii* differs from phagosomes in that it does not acquire the host derived proteins involved in endosome fusion thereby protecting the parasite from lysosomal destruction (Beyer *et al.*, 2002; Mordue *et al.*, 1999; Mordue & Sibley, 1997). Although assumed to be similar, the fusion capacities of the PV harbouring *Eimeria* species has not yet been demonstrated (Entzeroth *et al.*, 1998).

ii. Signalling pathways involved in invasion

Waves of regulated protein secretion from the microneme (MIC) and rhoptry (ROP/RON) apical organelles are essential for parasite movement, invasion, formation of the intracellular parasitophorous vacuole, control of host gene expression and egress of daughter zoites from infected cells. These processes are key virulence determinants for most species of the Apicomplexa (Besteiro *et al.*, 2011; Dubremetz *et al.*, 1998; Keeley & Soldati, 2004).

Rapid secretion and surface capping of microneme proteins (MICs) from the apical tip of *E. tenella* sporozoites is induced when sporozoites are allowed to glide over a substrate and during invasion of host cells in cell culture (Bumstead & Tomley, 2000). In the absence of host cells, secretion and capping can be induced by exposure of freshly purified sporozoites to serum, or purified albumin at temperatures of 37^{o}C or 41^{o}C (Brown *et al.*, 2000; Bumstead & Tomley, 2000). At lower temperatures or in the absence of serum or albumin, no MIC secretion or capping is detected. Both parasite invasion and albumin-induced MIC secretion is blocked in *E. tenella* by treatment with a compound that directly

inhibits protein kinase G (Wiersma *et al.*, 2004) indicating the likely importance of cyclic GMP (cGMP) signaling in coccidian secretion. PKG-dependent microneme secretion has also been shown in *T. gondii* (Brown et al., 2000) and using a novel auxin-inducible degron (AID) tagging system for conditional protein depletion in *T. gondii* alongside CRISPR-Cas9 genome editing, signaling was shown to go through PKG^{I}, a myristoylated isoform of PKG localized at the parasite plasma membrane (Brown *et al.*, 2017). In addition to cGMP signaling, it is known that calcium (Ca^{2+}) fluxes provide crucial signals for gliding motility, microneme secretion, conoid extrusion, invasion and egress (Lourido *et al.*, 2010; Pu & Zhang, 2012). These pathways operate through specific members of a calcium-dependent protein kinase (CDPK) family that is conserved in *Eimeria* (Dunn et al., 1996). A detailed chemical genetics (mutation) approach shows that the pathways linked to parasite invasion and egress, and the secretion of specific MIC proteins are differentially controlled by different CDPKs, and intersect cGMP signaling (Besteiro *et al.*, 2011; Lourido *et al.*, 2012). A variety of treatments that cause transient fluxes of cytosolic Ca^{2+} induce MIC secretion in *T. gondii* tachyzoites including calcium ionophores (Carruthers & Sibley, 1999), ethanol and acetaldehyde, but this is dependent upon the presence of albumin and cGMP signaling (Brown *et al.*, 2016). Similarly in *E. tenella*, acetaldehyde and ethanol stimulate Ca^{2+} dependent MIC secretion and premature egress of sporozoites from cultured cells in the presence of serum (Yan *et al.*, 2015; Yan *et al.*, 2016), however in the absence of serum or albumin neither ionophores nor ethanol/acetalydehyde are effective (F. M. Tomley and J.M. Bumstead, unpublished). Thus it appears that cGMP and Ca^{2+} signaling pathways work co-operatively in MIC signaling, with PKGI at the plasma membrane being the essential 'master' regulator (Brown *et al.*, 2017) whilst members of the CDPK family provide the selectivity and specificity needed to carry out specific biological functions such as invasion or egress.

Signaling pathways leading to the exocytosis of rhoptry contents are not yet defined however these must allow the selective secretion of RON proteins early in readiness for their role in formation of the MJ (Besteiro *et al.*, 2011). It has also been reported that *T. gondii* is able to inject ROP proteins into

host cells that it does not invade, allowing the parasite to manipulate uninfected cells (Koshy *et al.*, 2012) suggesting that e-vacuole (Hakansson *et al.*, 2001) deployment is an important virulence factor.

iii. The role of micronemes and the glideosome

Microneme proteins (MICs) are secreted from the parasite apex either singly or as protein complexes onto the parasite surface (Brown *et al.*, 2000; Bumstead & Tomley, 2000; Lai *et al.*, 2009; Tomley *et al.*, 1996) a process mediated in *T. gondii* by DOC2 proteins that recruit the necessary membrane-fusion machinery (Farrell *et al.*, 2012). 'Capping' models of motility, whereby parasite molecules are rapidly translocated backwards over the surface to promote forward motion, were proposed over 40 years ago for *Plasmodium* (circumsporozoite precipitation reaction, Vanderberg, 1974), *Eimeria nieschulzi* (capping of ferritin, Dubremetz & Torpier, 1978) and gregarines (capping of conA coated latex beads, King, 1981). The importance of the actin in motility was recognized in both *Eimeria* (Jensen & Edgar, 1976; Russell & Sinden, 1982) and *Plasmodium* (Miller *et al.*, 1979) and a later study in *E. tenella* showed that material secreted when sporozoites were allowed to glide on a substrate emanated from the apical tip (Entzeroth *et al.*, 1989). Subsequently, a large number of studies, mainly in *T. gondii*, has led to definition of 'glideosomes' (Opitz & Soldati, 2002), protein complexes that lie between the parasite plasma membrane and the IMC and power substrate-dependent gliding motility (reviewed in detail by Frenal & Soldati-Favre, 2009).

In brief, binding of surface-bound MIC adhesins to host ligands provides traction, linkage of these parasite-host surface membrane complexes to the underlying action-myosin motor is needed for their translocation (capping). This is achieved by the glideosome-associated connector (GAC), an armadillo repeat-containing protein that accumulates under the plasma membrane at the apical tip and stabilizes freshly polymerized short F-actin filaments that are nucleated at the tip by parasite formins (Jacot *et al.*, 2016). GAC binds directly to the cytosolic tails of surface-bound transmembrane MICs (Jacot *et al.*, 2016) and in a two stage process, stabilized actin-GAC-MIC complexes are rapidly shuttled

388 backwards through the interaction of the actin tracks initially with MyoH glideosomes, that are
389 restricted to the conoid region (Graindorge *et al.*, 2016), and thereafter with MyoA glideosomes
390 positioned along the length of the zoite (Herm-Gotz *et al.*, 2002). The complexes are shed from the
391 posterior of zoites by the action of an intramembrane rhomboid-like serine protease, ROM4, which
392 cleaves MIC transmembrane spanning regions (Buguliskis *et al.*, 2010). To generate the force needed
393 for forward motion, glideosomes need to be linked fluidly at the parasite plasma membrane and
394 immobilized onto the cytoskeleton, a feat achieved by glideosome protein GAP45 which has its
395 acylated N-terminus embedded in the plasma membrane and its C-terminus cross-linked to the IMC
396 (Gilk *et al.*, 2009). Additional glideosome proteins GAP40 and GAP50 are further involved in anchoring
397 MyoA firmly the IMC (Harding *et al.*, 2016) and a family of multi-membrane spanning GAPM proteins
398 connect the glideosome right through to the subpellicular microtubules, via interaction with alveolins
399 (Bullen *et al.*, 2009). The regular positioning and complex molecular architecture of the glideosomes
400 suggests that these structures are equivalent to the intramembrane particles visible in scanning
401 electron micrographs of freeze-fractured IMC from sporozoites of *Eimeria* taken over 40 years ago
402 (Dubremetz & Torpier, 1978).

403 By virtue of their host-binding activity, MICs are major contributors to parasite host-range and
404 specificity; for example, MAR (microneme adhesive repeat)-domain containing MICs of *E. tenella*
405 contain a single type (type 1) of MAR (Lai *et al.*, 2011) whereas *T. gondii* and *N. caninum* possess MICs
406 with both type 1 and 2 MAR. MARs bind sialyl-terminated oligosaccharides from many types of
407 vertebrate tissue so expressing only a single type effectively narrows the range of sialylated receptors
408 that *E. tenella* can bind, contributing to the very specific tropism of this parasite (Cowper *et al.*, 2012).
409 In *T. gondii* secretion of perforin from micronemes is essential for tachyzoite egress from vacuoles
410 (Roiko & Carruthers, 2013). A role for perforin in egress has not been confirmed for *Eimeria* parasites;
411 a gene encoding a conserved membrane-attack complex/perforin is expressed in *E. tenella* sporozoites

but appears to be down-regulated in the later merozoite and gamete stages (Reid *et al.*, 2014; Walker *et al.*, 2015).

iv. Secretion of rhoptries and dense granules proteins

RONs (rhoptry neck protein) and ROPs (rhoptry bulb proteins) are believed to discharge from the apex of the parasite; rhoptry ducts run through the conoid and terminate at the very apical tip. In *T. gondii,* RONs act in concert with apical membrane antigens (AMA, secreted from the micronemes) at the early stage of invasion, assembling at the parasite-host interface to form the irreversible MJ (Besteiro *et al.*, 2011; Lamarque *et al.*, 2011; Tyler & Boothroyd, 2011) actively recruiting host proteins to the MJ, subverting their function to enhance invasion efficiency (Guerin *et al.*, 2017). Proteomic and genomic analysis readily identified several families of RONs in *E. tenella* orthologous to those of *T.gondii*, along with stage-regulated expression of specific AMA and EtRON2 family members (Oakes *et al.*, 2013), suggesting that the mechanism by which the MJ is built by different coccidians is conserved. In contrast there is only limited conservation of ROPs between *T. gondii* and *E. tenella* including significant divergence in the families of ROP kinases that are the major component of the ROP proteome (Oakes *et al.*, 2013; Talevich & Kannan, 2013) and which are known to be key virulence factors in *T. gondii*. Little is known of the specific function of individual ROP proteins in *Eimeria.* Among the *Eimeria* species affecting chickens, rhoptry proteins offer little immunological cross-reactivity between the various species or even between different life cycle stages within the same species (Kawazoe *et al.*, 1992; Tomley, 1994).

Another group of secretory organelles related to host cell interactions in *T. gondii*, and other heteroxenic, cyst-forming coccidian, are the dense granules. These are roughly spherical structures larger than micronemes but smaller than rhoptries (Paredes-Santos *et al.*, 2012). The contents of dense granules are secreted into the PV during and immediately after invasion, and dense granule proteins (GRA) are targeted to a variety of final locations including the PV cavity, PV membrane, host

cell cytoplasm and host cell nucleolus (Mercier & Cesbron-Delauw, 2015). However, the presence of dense granules as an independent organelle in the zoites of *Eimeria* spp. is uncertain as there is a lack of structural evidence (Entzeroth *et al.*, 1998; Vetterling *et al.*, 1973) and moreover only a very small number of GRA orthologues are found in their genomes (Reid *et al.*, 2014).

v. Post-invasion events

For *Eimeria* species, the newly-formed PV is small and closely surrounds the parasite but later enlarges, possibly contain membranous material or projections from the vacuolar membrane (Lee & Long, 1972; Pacheco *et al.*, 1975; Strout & Scholtyseck, 1970; Vetterling *et al.*, 1973). In electron micrographs of recently invaded sporozoites and merozoites, the PV is often not visible. It is unclear whether this is because there is no vacuole present or because the vacuole membrane is so closely opposed to the parasite that it cannot be distinguished (Lee & Long, 1972; McLaren, 1969; Mota & Rodriguez, 2001). Between 24 and 35 hours after invasion, the intracellular sporozoite becomes ovoid in shape (Fig. 6.1). At this stage, the parasite is known as a trophozoite and loses most of its apical complex and inner membrane (McLaren, 1969; Pacheco *et al.*, 1975).

Fairly soon following inoculation of *in vitro* cell cultures, some *Eimeria* species sporozoites have been seen to leave their invaded host cell without undergoing further development and replication. It has been hypothesised that some of this cell traversal may involve penetration through the host cell plasma membrane rather than formation of a PV (Behrendt *et al.*, 2004; Itagaki *et al.*, 1974). This hypothesis is supported by the observation that *Plasmodium yoelii* sporozoites will sometimes invade hepatocytes by breaching the host cell membrane (Mota *et al.*, 2001). Breaching of the host cell membrane has been described for *E. bovis* (Behrendt *et al.*, 2004), however studies using *E. magna* demonstrated no breach to plasma membrane on parasite invasion (Jensen, 1975; Jensen & Edgar, 1976). This suggests that invasion of cells by *Eimeria* species does indeed follow the generally accepted model for apicomplexan invasion.

Initial invasion of *E. acervulina, E. maxima, E. necatrix*, and *E. tenella in vivo* occurs at the villus epithelium (Lillehoj & Trout, 1993; Shirley *et al.*, 2005). Before initiating endogenous development however, these species travel to the intestinal crypts where they invade another cell of the intestinal epithelium (Jeurissen *et al.*, 1996; Shirley *et al.*, 2005). Although the mechanism by which sporozoites travel from the villi to the crypts is not fully understood, it is believed to occur through the interaction with intestinal lymphocytes (Lawn & Rose, 1982). There are some species of *Eimeria* whose life cycles involve migration out of the gastrointestinal tract. Sporozoites of the rabbit coccidium, *Eimeria stiedae,* migrate from the duodenum to the liver (Pakandl, 2009). Two species of *Eimeria* which infect cranes, *E. reichenowi* and *E. gruis,* produce a disease known as disseminated visceral coccidiosis, where zoites can be found in diverse organs such as the lungs, liver and heart (Novilla & Carpenter, 2004). The specific mechanisms involved in this type of migration are not fully understood; traffic via portal vein as well as the lymphatic system was an initial hypothesis (Fitzgerald, 1970); alternatively, spread throughout the host organism until settle in the liver has been suggested (Durr, 1972).

Schizogony

The next phase of development in *Eimeria* species consists of two to five rounds of asexual replication known as schizogony, where nuclear divisions and cellular expansion occurs to produce a multinuclear schizont (Figs. 2.4, 2.5 and 6.2). The number of rounds of schizogony, the number of nuclear divisions, and the specific site of development are specific characteristic to each species of *Eimeria* parasite. *Eimeria tenella* has three generations of schizogony, all located in the caecal crypts, whereas *E. maxima* has four-to-five generations mostly located in the villi of the small intestine (Dubey & Jenkins, 2018; McDonald & Rose, 1987). In the early stages of schizogony (up to 35 hours post invasion) proliferation of the parasite endoplasmic reticulum occurs and nuclear divisions result in multiple granular nuclei, each enclosed by a perforated double membrane. During these divisions, intranuclear spindles, centrocones and centrioles can all be seen (Pacheco *et al.*, 1975). Centrioles of *Eimeria* have a 9+1 singlet microtubule pattern, as opposed to the nine triplet symmetry found in mammalian cells

(Dubremetz & Elsner, 1979). As the replicating schizont forms, the parasite significantly increases in size and may occupy up to half of the host cell content (McLaren, 1969).

After nuclear division, individual merozoites begin to develop in the form of protrusions of the schizont cytoplasm that develop a conoid at the apex (Fig. 6.3). Each merozoite then elongates and receives a single nucleus from the schizont. The mitochondria and apicoplasts (non-photosynthetic plastid organelle) of zoites of *Eimeria* and other coccidian species contain nucleic acid genomes that must be replicated and segregated into each of the forming merozoites. Apicoplast replication in *Eimeria* species occurs via a different mechanism to that in *T. gondii* tachyzoites, which divide by endodyogeny (rather than schizogony) with apicoplasts dividing in close association with centrosomes and in synchrony with nuclear division (Striepen *et al.*, 2000). In *E. tenella*, over 95% of sporozoites contain a single apicoplast with up to 5% having two or three of them, whereas to 20% of merozoites have multiple apicoplasts (Ferguson *et al.*, 2007). After zoite invasion, *E. tenella* apicoplasts enlarge to form pleomorphic-shaped structures that divide several times during the proliferative phase of schizogony. This is not associated with centrosomes and occurs independently of nuclear division by an unknown mechanism (Ferguson *et al.*, 2007). Correct segregation of daughter mitochondria during schizogony is also poorly understood. Once schizonts are fully formed, the posterior poles of merozoites undergo constriction by cytoskeletal rings until they separate from what remains of the schizont, known as the residual body (McLaren, 1969; Pacheco *et al.*, 1975). The events that occur during the second round of schizogony in *E. tenella* and *E. necatrix* are particularly interesting since parasite infection causes the host cells to detach from adjacent cells and migrate deeper into the underlying tissue (del Cacho *et al.*, 2004; Fernando *et al.*, 1983; Stockdale & Fernando, 1975). In *E. tenella*, the 2nd generation schizonts are larger than 1st generation schizonts, and the 3rd generation schizonts are significantly smaller, containing less than 16 merozoites per structure (Lee & Long, 1972; McDonald & Rose, 1987; McLaren & Paget, 1968).

509 Merozoites share many characteristics and features of the sporozoite (Fig. 5.C). They are bound by a
510 triple bilayer pellicle (plasma membrane and double-layered IMC) and contain a posteriorly located
511 nucleus of similar appearance to that of the sporozoite (McLaren & Paget, 1968). Amylopectin
512 granules, endoplasmic reticulum, Golgi, and an apical complex are also present; however they do not
513 contain refractile bodies, just a refractile dot. Second generation merozoites have been reported to
514 have more micronemes but fewer rhoptries than their sporozoite counterparts (McLaren & Paget,
515 1968; Pacheco *et al.*, 1975). A couple of 'extra structures' have also been reported in newly formed
516 merozoites, namely rod-shaped mitochondria and a vacuole with an electron dense outer membrane
517 (McLaren, 1969).

518 *Gametogony and fertilization*

519 Upon re-invasion, the final generation of merozoites initiates a single round of sexual replication,
520 however due to limitations of *in vitro* development little is known about this stage in *Eimeria* species.
521 Despite some studies reporting a complete reproduction of the life cycle *in vitro*, this system is still
522 very deficient, meaning that investigation of the sexual stages requires the use of a host animal
523 (Hermosilla *et al.*, 2002). Microscopy of tissue infected *in vivo*, revealed that *Eimeria* species develop
524 two sexually dimorphic stages; the macrogamete and the microgamete (Walker *et al.*, 2013).
525 Macrogametes are large cells, measuring over 9µm by 16µm, and contain numerous polysaccharide
526 storage granules for providing nutrients to the developing oocyst (McLaren, 1969). They also contain
527 multiple structures known as wall forming bodies and veil forming bodies which are important for
528 production of the oocyst wall (Ferguson *et al.*, 2003). Microgametes are considerably smaller, around
529 0.5µm by 5µm, and possess two flagella which enhances their motility needed for reaching and
530 fertilising a macrogamete (Madden & Vetterling, 1977). The formation of microgametes occurs in a
531 similar way to the formation of merozoites by schizogony. Multiple nuclear divisions are performed
532 followed by differentiation of mature flagellated microgametes, roughly 100 from each initial cell. This
533 is markedly different from the process of microgamete formation that occurs in the haemosporines

(including *Plasmodium* species) where microgametogenesis occurs as a result of chemical cues from the insect vector and involves extremely rapid exflagellation (the whole process taking around 8-15minutes) (Billker *et al.*, 1998; Sinden & Croll, 1975).

Fertilization of the macrogamete by a microgamete results in the formation of the zygote, which is encased by the forming oocyst wall prior to excretion with the faeces (Fig. 2.6 - 2.7) (Jeurissen *et al.*, 1996; Shirley *et al.*, 2005). Ferguson *et al.* examined the ultrastructure of *T. gondii* microgametes (Ferguson *et al.*, 1974): these contain a dense nucleus, a single mitochondrion, two flagella which arise from basal bodies located within the cytoplasm and an osmophilic plate under the plasma membrane at the anterior of the cell. It was also observed that the number of microgametes produced were much lower than expected, meaning that there is no room for wastage if every macrogamete is to be fertilised (Ferguson, 2002). Two hypotheses have been presented to explain this phenomenon: 1. viable oocysts can be produced in absence of fertilisation, and 2. the adaptive sex ratio theory, where, due to the high likelihood of inbreeding, selection pressure leads to production of only the minimal number of microgametes required for fertilisation of the macrogametes (Ferguson, 2002; West *et al.*, 2003).

Parasite manipulation of the host cell

With most of their development occurring within a vacuole in the cytoplasm of another cell, coccidian parasites have an intimate relationship with the host (Jeurissen *et al.*, 1996). *Eimeria tenella* is incapable of *de novo* synthesis of purines and therefore must salvage these in a pre-formed state, relying on the host metabolism for this compound (LaFon & Nelson, 1985). With *E. bovis*, infection has been shown to significantly modify the host cell, altering gene expression relating to cell metabolism, cell structure, protein synthesis and gene transcription, suggesting that the parasite is able to manipulate the host cell in multiple ways that are advantageous to its survival (Lutz *et al.*, 2011). In *T. gondii* there is evidence that the parasite uses multiple mechanisms to intercept the normal apoptotic

pathways of the host cell and thereby prevent destruction of its immediate environment. One such mechanism used by *T. gondii* involves activation of the transcription factor nuclear factor kB (NF-kB). Results of immune-histochemical staining of parasitized chicken tissue, suggest that this pathway is also utilised by species of *Eimeria* in avoiding host cell apoptosis (del Cacho *et al.*, 2004). The intracellular development of *E. bovis* is particularly slow, compared to other *Eimeria* species, taking around two weeks to complete the first round of replication, forming exceptionally large schizonts (300µm) known as macromeronts (Lutz *et al.*, 2011). In order to maintain host cell viability for this time, despite the pressures of parasitism, it seems especially likely that *E. bovis* is able to disrupt the apoptotic pathways of the host; indeed in cultured cells heavily infected by *E. bovis,* it is ultimately the uninfected cells which are seen to die off, whilst the infected cells survive (Lang *et al.*, 2009). These infected cells were shown to have increased expression of anti-apoptotic factors such as cellular Flice inhibitory protein (c-FLIP) and cellular inhibition of apoptosis protein 1 (c-IAP1).

DISCUSSION

Parasites of the *Eimeria* genus are highly complex organisms, containing numerous structures and exhibiting complex life cycle and processes, some of which are markedly different to higher eukaryotes. Regardless of their high impact and wide prevalence, there are many mechanisms and morphological features that remain completely uncharacterised in *Eimeria* spp. Although *T. gondii* is an invaluable resource for inferring information, in particular regarding early endogenous replication, where proteins from specific secretory organelles (micronemes, rhoptries and dense granules) are essential for attachment, invasion, formation and modification of the intracellular parasitophorous vacuole and modulation of host cell pathways; it is a different organism and the pathogenesis of the two parasites differs slightly. Whereas the mechanisms of invasion are similar, the intracellular development is significantly different. *Eimeria* has an acute, monoxenous life cycle, with no parasite stages persist within host tissue. In the other hand, *T. gondii* has a heteroxenous life cycle with acute and chronic phases; when ingested by intermediate hosts, parasites transform into tachyzoites that

583 are found transiently in many tissues before they migrate to neural and muscle tissues, where they
584 convert to the tissue cyst bradyzoites that remain in host tissues for life.

585 Therefore, potential targets and strategies for the control of toxoplasmosis would differ to those to
586 control coccidiosis. For example, previous work on genomics and proteomics of *E. tenella* (Oakes *et*
587 *al*., 2013; Reid *et al*., 2014) have shown an excellent conservation between *Eimeria* and *Toxoplasma*
588 micronemes and rhoptry neck proteins involved in the first stages of endogenous development.
589 However, there is much more limited conservation of rhoptry bulb proteins. In addition, there is very
590 little conservation of genes encoding dense granules proteins (GRA). The different aim of the
591 parasitaphorous vacuole created by sporozoites (residing for short term leading to sexual
592 reproduction) *versus* tachyzoites (establishing a chronic infection, eventually) could be one of the
593 answers to this variable composition in ROPs and GRAs. This together with the lack of electron
594 microscope evidence raise the significant question of whether *Eimeria* parasites contain organelles
595 equivalent to dense granules of *Toxoplasma* and other cyst-forming coccidian. It is also interesting
596 that the largest organelle of the parasite cell (refractile bodies) with potential compounds that could
597 serve as a target for disease control, still have an undetermined function.

598 Since the boom of molecular biology towards the end of the 20th century, there has been a decrease
599 in microscopy-led biological research. However, both light and electron microscopes are invaluable
600 tools for studying organisms such as *Eimeria*, which have limited *in vitro* systems and molecular tools
601 for gene editing. For many biological structures, morphology is closely linked to function. Microscopy-
602 derived data can therefore help answer questions about the function of subcellular structures and
603 even individual proteins. In organisms with extensive genetic toolkits (such as *T. gondii*. or
604 *Trypanosoma* spp.) microscopy can still be used to determine protein location and function, following
605 the use of fluorescent tagging and protein synthesis disruption. As development of genetic techniques
606 for species of *Eimeria* progresses, it is likely that these techniques will also play an important part in
607 unravelling the biology of this species. Additionally, advances in cell culture systems and in genetic

modification tools for *Eimeria* species (e.g. CRISPR/Cas9) could play an important role to answer some of the many questions regarding the functions and properties of eimerian subcellular structures and organelles such as the refractile bodies, secretory organelles, apicoplast and conoid, each of which could potentially contain molecules that for targeting by novel drugs due to their absence in the cells of higher eukaryote host species.

CONCLUSIONS/FUTURE DIRECTIONS

In this paper we have reviewed what is known about the life cycle and developmental stages of members of the *Eimeria* genus. An overarching aim in apicomplexan disease research is the production of affordable and sustainable vaccines and there is therefore a wealth of studies focused on the identification and testing of possible immunoprotective antigens. However, the identification of new candidates will not be possible without a complete understanding of eimerian biology. Investment in *in vitro* systems to get further in the parasite life cycle and testing alternative compound to control the disease are paramount, together with the development of new molecular tools for gene edition in *Eimeria* spp.

FINANCIAL SUPPORT

Alana Burrell was funded by the RVC PhD studentship programme. This manuscript has been assigned the reference PPS_02029 by the Royal Veterinary College.

625 REFERENCES

626 **Adl, S. M., Leander, B. S., Simpson, A. G., Archibald, J. M., Anderson, O. R., Bass, D., Bowser, S. S.,**
627 **Brugerolle, G., Farmer, M. A., Karpov, S., Kolisko, M., Lane, C. E., Lodge, D. J., Mann, D. G.,**
628 **Meisterfeld, R., Mendoza, L., Moestrup, O., Mozley-Standridge, S. E., Smirnov, A. V. and Spiegel, F.**
629 (2007). Diversity, nomenclature, and taxonomy of protists. *Syst Biol,* **56**, 684-689. doi:
630 10.1080/10635150701494127.

631 **Aikawa, M., Miller, L. H., Johnson, J. and Rabbege, J.** (1978). Erythrocyte entry by malarial parasites.
632 A moving junction between erythrocyte and parasite. *J Cell Biol,* **77**, 72-82. doi: 10.1083/jcb.77.1.72.

633 **Augustine, P. C.** (2001). Cell: sporozoite interactions and invasion by apicomplexan parasites of the
634 genus Eimeria. *Int J Parasitol,* **31**, 1-8.

635 **Behrendt, J. H., Clauss, W., Zahner, H. and Hermosilla, C.** (2004). Alternative mechanism of Eimeria
636 bovis sporozoites to invade cells in vitro by breaching the plasma membrane. *J Parasitol,* **90**, 1163-
637 1165. doi: 10.1645/GE-3285RN.

638 **Belli, S. I., Smith, N. C. and Ferguson, D. J.** (2006). The coccidian oocyst: a tough nut to crack! *Trends*
639 *Parasitol,* **22**, 416-423. doi: 10.1016/j.pt.2006.07.004.

640 **Besteiro, S., Dubremetz, J. F. and Lebrun, M.** (2011). The moving junction of apicomplexan parasites:
641 a key structure for invasion. *Cell Microbiol,* **13**, 797-805. doi: 10.1111/j.1462-5822.2011.01597.x.

642 **Beyer, T. V., Svezhova, N. V., Radchenko, A. I. and Sidorenko, N. V.** (2002). Parasitophorous vacuole:
643 morphofunctional diversity in different coccidian genera (a short insight into the problem). *Cell Biol*
644 *Int,* **26**, 861-871.

645 **Bhogal, B. S., Miller, G. A., Anderson, A. C., Jessee, E. J., Strausberg, R., McCandliss, R. and**
646 **Strausberg, S.** (1989). Vaccination of chickens with recombinant E. tenella antigen alone or in

combination with a subclinical exposure induces cross protective immunity against coccidiosis. *Prog Clin Biol Res,* **307**, 131-146.

Billker, O., Lindo, V., Panico, M., Etienne, A. E., Paxton, T., Dell, A., Rogers, M., Sinden, R. E. and Morris, H. R. (1998). Identification of xanthurenic acid as the putative inducer of malaria development in the mosquito. *Nature,* **392**, 289-292. doi: 10.1038/32667.

Bommer, W., Heunert, H. H. and Milthaler, B. (1969). [Cinematographic studies on the movement of Toxoplasma gondii]. *Z Tropenmed Parasitol,* **20**, 450-458.

Bonifacino, J. S. and Rojas, R. (2006). Retrograde transport from endosomes to the trans-Golgi network. *Nat Rev Mol Cell Biol,* **7**, 568-579. doi: 10.1038/nrm1985.

Breinich, M. S., Ferguson, D. J., Foth, B. J., van Dooren, G. G., Lebrun, M., Quon, D. V., Striepen, B., Bradley, P. J., Frischknecht, F., Carruthers, V. B. and Meissner, M. (2009). A dynamin is required for the biogenesis of secretory organelles in Toxoplasma gondii. *Curr Biol,* **19**, 277-286. doi: 10.1016/j.cub.2009.01.039.

Brown, K. M., Long, S. and Sibley, L. D. (2017). Plasma Membrane Association by N-Acylation Governs PKG Function in Toxoplasma gondii. *MBio,* **8**. doi: 10.1128/mBio.00375-17.

Brown, K. M., Lourido, S. and Sibley, L. D. (2016). Serum Albumin Stimulates Protein Kinase G-dependent Microneme Secretion in Toxoplasma gondii. *J Biol Chem,* **291**, 9554-9565. doi: 10.1074/jbc.M115.700518.

Brown, P. J., Billington, K. J., Bumstead, J. M., Clark, J. D. and Tomley, F. M. (2000). A microneme protein from Eimeria tenella with homology to the Apple domains of coagulation factor XI and plasma pre-kallikrein. *Mol Biochem Parasitol,* **107**, 91-102.

668 **Buguliskis, J. S., Brossier, F., Shuman, J. and Sibley, L. D.** (2010). Rhomboid 4 (ROM4) affects the
669 processing of surface adhesins and facilitates host cell invasion by Toxoplasma gondii. *PLoS Pathog,* **6**,
670 e1000858. doi: 10.1371/journal.ppat.1000858.

671 **Bullen, H. E., Tonkin, C. J., O'Donnell, R. A., Tham, W. H., Papenfuss, A. T., Gould, S., Cowman, A. F.,**
672 **Crabb, B. S. and Gilson, P. R.** (2009). A novel family of Apicomplexan glideosome-associated proteins
673 with an inner membrane-anchoring role. *J Biol Chem,* **284**, 25353-25363. doi:
674 10.1074/jbc.M109.036772.

675 **Bumstead, J. and Tomley, F.** (2000). Induction of secretion and surface capping of microneme proteins
676 in Eimeria tenella. *Mol Biochem Parasitol,* **110**, 311-321.

677 **Canning, E. U. and Anwar, M.** (1968). Studies on meiotic division in coccidial and malarial parasites. *J*
678 *Protozool,* **15**, 290-298.

679 **Carreno, R. A., Martin, D. S. and Barta, J. R.** (1999). Cryptosporidium is more closely related to the
680 gregarines than to coccidia as shown by phylogenetic analysis of apicomplexan parasites inferred using
681 small-subunit ribosomal RNA gene sequences. *Parasitol Res,* **85**, 899-904.

682 **Carruthers, V. and Boothroyd, J. C.** (2007). Pulling together: an integrated model of Toxoplasma cell
683 invasion. *Curr Opin Microbiol,* **10**, 83-89. doi: 10.1016/j.mib.2006.06.017.

684 **Carruthers, V. B. and Sibley, L. D.** (1999). Mobilization of intracellular calcium stimulates microneme
685 discharge in Toxoplasma gondii. *Mol Microbiol,* **31**, 421-428. doi: 10.1046/j.1365-2958.1999.01174.x.

686 **Carruthers, V. B. and Tomley, F. M.** (2008). Microneme proteins in apicomplexans. *Subcell Biochem,*
687 **47**, 33-45.

688 **Chiappino, M. L., Nichols, B. A. and O'Connor, G. R.** (1984). Scanning electron microscopy of
689 Toxoplasma gondii: parasite torsion and host-cell responses during invasion. *J Protozool,* **31**, 288-292.

690 **Clark, E. L., Macdonald, S. E., Thenmozhi, V., Kundu, K., Garg, R., Kumar, S., Ayoade, S., Fornace, K.**
691 **M., Jatau, I. D., Moftah, A., Nolan, M. J., Sudhakar, N. R., Adebambo, A. O., Lawal, I. A., Alvarez**
692 **Zapata, R., Awuni, J. A., Chapman, H. D., Karimuribo, E., Mugasa, C. M., Namangala, B., Rushton, J.,**
693 **Suo, X., Thangaraj, K., Srinivasa Rao, A. S., Tewari, A. K., Banerjee, P. S., Dhinakar Raj, G., Raman,**
694 **M., Tomley, F. M. and Blake, D. P.** (2016). Cryptic Eimeria genotypes are common across the southern
695 but not northern hemisphere. *Int J Parasitol,* **46**, 537-544. doi: 10.1016/j.ijpara.2016.05.006.

696 **Coombs, G. H., Denton, H., Brown, S. M. and Thong, K. W.** (1997). Biochemistry of the coccidia. *Adv*
697 *Parasitol,* **39**, 141-226.

698 **Cowper, B., Matthews, S. and Tomley, F.** (2012). The molecular basis for the distinct host and tissue
699 tropisms of coccidian parasites. *Mol Biochem Parasitol,* **186**, 1-10. doi:
700 10.1016/j.molbiopara.2012.08.007.

701 **Crane, M. S., Goggin, B., Pellegrino, R. M., Ravino, O. J., Lange, C., Karkhanis, Y. D., Kirk, K. E. and**
702 **Chakraborty, P. R.** (1991). Cross-protection against four species of chicken coccidia with a single
703 recombinant antigen. *Infect Immun,* **59**, 1271-1277.

704 **Danforth, H. D. and Augustine, P. C.** (1989). Eimeria tenella: use of a monoclonal antibody in
705 determining the intracellular fate of the refractile body organelles and the effect on in vitro
706 development. *Exp Parasitol,* **68**, 1-7.

707 **de Venevelles, P., Francois Chich, J., Faigle, W., Lombard, B., Loew, D., Pery, P. and Labbe, M.** (2006).
708 Study of proteins associated with the Eimeria tenella refractile body by a proteomic approach. *Int J*
709 *Parasitol,* **36**, 1399-1407. doi: 10.1016/j.ijpara.2006.06.018.

710 **del Cacho, E., Gallego, M., Lopez-Bernad, F., Quilez, J. and Sanchez-Acedo, C.** (2004). Expression of
711 anti-apoptotic factors in cells parasitized by second-generation schizonts of Eimeria tenella and
712 Eimeria necatrix. *Vet Parasitol,* **125**, 287-300. doi: 10.1016/j.vetpar.2004.07.017.

713 **Dobell, C.** (1922). The discovery of the coccidia. *Parasitololy,* **14**, 342-348.

714 **Dogga, S. K., Mukherjee, B., Jacot, D., Kockmann, T., Molino, L., Hammoudi, P. M., Hartkoorn, R. C.,**
715 **Hehl, A. B. and Soldati-Favre, D.** (2017). A druggable secretory protein maturase of Toxoplasma
716 essential for invasion and egress. *Elife,* **6**. doi: 10.7554/eLife.27480.

717 **Dou, Z., McGovern, O. L., Di Cristina, M. and Carruthers, V. B.** (2014). Toxoplasma gondii ingests and
718 digests host cytosolic proteins. *MBio,* **5**, e01188-01114. doi: 10.1128/mBio.01188-14.

719 **Dubey, J. P. and Jenkins, M. C.** (2018). Re-evaluation of the life cycle of Eimeria maxima Tyzzer, 1929
720 in chickens (Gallus domesticus). *Parasitology,* **145**, 1051-1058. doi: 10.1017/S0031182017002153.

721 **Dubey, J. P., Lindsay, D. S. and Speer, C. A.** (1998). Structures of Toxoplasma gondii tachyzoites,
722 bradyzoites, and sporozoites and biology and development of tissue cysts. *Clin Microbiol Rev,* **11**, 267-
723 299.

724 **Dubremetz, J. F.** (1975). [Genesis of merozoites in the coccidia, Eimeria necatrix. Ultrastructural
725 study]. *J Protozool,* **22**, 71-84.

726 **Dubremetz, J. F. and Elsner, Y. Y.** (1979). Ultrastructural study of schizogony of Eimeria bovis in cell
727 cultures. *J Protozool,* **26**, 367-376.

728 **Dubremetz, J. F., Garcia-Reguet, N., Conseil, V. and Fourmaux, M. N.** (1998). Apical organelles and
729 host-cell invasion by Apicomplexa. *Int J Parasitol,* **28**, 1007-1013.

730 **Dubremetz, J. F. and Torpier, G.** (1978). Freeze fracture study of the pellicle of an eimerian sporozoite
731 (Protozoa, Coccidia). *J Ultrastruct Res,* **62**, 94-109.

Dunn, P. P., Bumstead, J. M. and Tomley, F. M. (1996). Sequence, expression and localization of calmodulin-domain protein kinases in Eimeria tenella and Eimeria maxima. *Parasitology,* **113 (Pt 5)**, 439-448.

Durr, U. (1972). Life cycle of Eimeria stiedai. *Acta Vet Acad Sci Hung,* **22**, 101-103.

Duszynski, D.W. (2001). *Eimeria*, In: eLS. John Wiley & Sons, Ltd.

Edgar, S. A. and Seibold, C. T. (1964). A New Coccidium of Chickens, Eimeria Mivati Sp. N. (Protozoa: Eimeriidae) with Details of Its Life History. *J Parasitol,* **50**, 193-204.

Entzeroth, R., Mattig, F. R. and Werner-Meier, R. (1998). Structure and function of the parasitophorous vacuole in Eimeria species. *Int J Parasitol,* **28**, 1015-1018.

Entzeroth, R., Zgrzebski, G. and Dubremetz, J. F. (1989). Secretion of trials during gliding motility of Eimeria nieschulzi (Apicomplexa, Coccidia) sporozoites visualized by a monoclonal antibody and immuno-gold-silver enhancement. *Parasitol Res,* **76**, 174-175.

Fantham, H. (1910). The Morphology and Life-History of *Eimeria* (Coccidium) avium: a Sporozoö causing a fatal disease among young Grouse. In: *Proceedings of the Zoological Society of London*, pp. 672-691.

Farrell, A., Thirugnanam, S., Lorestani, A., Dvorin, J. D., Eidell, K. P., Ferguson, D. J., Anderson-White, B. R., Duraisingh, M. T., Marth, G. T. and Gubbels, M. J. (2012). A DOC2 protein identified by mutational profiling is essential for apicomplexan parasite exocytosis. *Science,* **335**, 218-221. doi: 10.1126/science.1210829.

Fayer, R. (1969). Refractile body changes in sporozoites of poultry coccidia in cell culture. In: Proc. Helm. Soc. Wash, pp. 224-231.

753 **Ferguson, D. J.** (2002). Toxoplasma gondii and sex: essential or optional extra? *Trends Parasitol,* **18**,
754 355-359.

755 **Ferguson, D. J., Belli, S. I., Smith, N. C. and Wallach, M. G.** (2003). The development of the
756 macrogamete and oocyst wall in Eimeria maxima: immuno-light and electron microscopy. *Int J*
757 *Parasitol,* **33**, 1329-1340.

758 **Ferguson, D. J., Campbell, S. A., Henriquez, F. L., Phan, L., Mui, E., Richards, T. A., Muench, S. P.,**
759 **Allary, M., Lu, J. Z., Prigge, S. T., Tomley, F., Shirley, M. W., Rice, D. W., McLeod, R. and Roberts, C.**
760 **W.** (2007). Enzymes of type II fatty acid synthesis and apicoplast differentiation and division in Eimeria
761 tenella. *Int J Parasitol,* **37**, 33-51. doi: 10.1016/j.ijpara.2006.10.003.

762 **Ferguson, D. J., Hutchison, W. M., Dunachie, J. F. and Siim, J. C.** (1974). Ultrastructural study of early
763 stages of asexual multiplication and microgametogony of Toxoplasma gondii in the small intestine of
764 the cat. *Acta Pathol Microbiol Scand B Microbiol Immunol,* **82**, 167-181.

765 **Fernando, M. A., Lawn, A. M., Rose, M. E. and Al-Attar, M. A.** (1983). Invasion of chicken caecal and
766 intestinal lamina propria by crypt epithelial cells infected with coccidia. *Parasitology,* **86 (Pt 3)**, 391-
767 398.

768 **Fernando, M. A. and Remmler, O.** (1974). Fine structure of the first and second generation merozoites
769 of Eimeria necatrix. *Z Parasitenkd,* **44**, 133-137.

770 **Fitzgerald, P.R.** (1970). New findings on the life cycle of Eimeria stiedae. *J. Parasitol*, **56**, 100–101.

771 **Francia, M. E. and Striepen, B.** (2014). Cell division in apicomplexan parasites. *Nat Rev Microbiol,* **12**,
772 125-136. doi: 10.1038/nrmicro3184.

773 **Frenal, K. and Soldati-Favre, D.** (2009). Role of the parasite and host cytoskeleton in apicomplexa
774 parasitism. *Cell Host Microbe,* **5**, 602-611. doi: 10.1016/j.chom.2009.05.013.

Gilk, S. D., Gaskins, E., Ward, G. E. and Beckers, C. J. (2009). GAP45 phosphorylation controls assembly of the Toxoplasma myosin XIV complex. *Eukaryot Cell,* **8**, 190-196. doi: 10.1128/EC.00201-08.

Golemansky, V. (2015). Checklist of Haemosporidian and Piroplasmid Parasites (Apicomplexa: Haemospororida and Pirolasmorida) of Man and Animals in Bulgaria. *Acta Zoologica Bulgarica*, **67**, 453-460.

Graindorge, A., Frenal, K., Jacot, D., Salamun, J., Marq, J. B. and Soldati-Favre, D. (2016). The Conoid Associated Motor MyoH Is Indispensable for Toxoplasma gondii Entry and Exit from Host Cells. *PLoS Pathog,* **12**, e1005388. doi: 10.1371/journal.ppat.1005388.

Guerardel, Y., Leleu, D., Coppin, A., Lienard, L., Slomianny, C., Strecker, G., Ball, S. and Tomavo, S. (2005). Amylopectin biogenesis and characterization in the protozoan parasite Toxoplasma gondii, the intracellular development of which is restricted in the HepG2 cell line. *Microbes Infect,* **7**, 41-48. doi: 10.1016/j.micinf.2004.09.007.

Guerin, A., Corrales, R. M., Parker, M. L., Lamarque, M. H., Jacot, D., El Hajj, H., Soldati-Favre, D., Boulanger, M. J. and Lebrun, M. (2017). Efficient invasion by Toxoplasma depends on the subversion of host protein networks. *Nat Microbiol,* **2**, 1358-1366. doi: 10.1038/s41564-017-0018-1.

Hakansson, S., Charron, A. J. and Sibley, L. D. (2001). Toxoplasma evacuoles: a two-step process of secretion and fusion forms the parasitophorous vacuole. *EMBO J,* **20**, 3132-3144. doi: 10.1093/emboj/20.12.3132.

Hammoudi, P. M., Maco, B., Dogga, S. K., Frenal, K. and Soldati-Favre, D. (2018). Toxoplasma gondii TFP1 is an essential transporter family protein critical for microneme maturation and exocytosis. *Mol Microbiol*. doi: 10.1111/mmi.13981.

796 **Harding, C. R., Egarter, S., Gow, M., Jimenez-Ruiz, E., Ferguson, D. J. and Meissner, M.** (2016). Gliding
797 Associated Proteins Play Essential Roles during the Formation of the Inner Membrane Complex of
798 Toxoplasma gondii. *PLoS Pathog,* **12**, e1005403. doi: 10.1371/journal.ppat.1005403.

799 **Herm-Gotz, A., Weiss, S., Stratmann, R., Fujita-Becker, S., Ruff, C., Meyhofer, E., Soldati, T.,**
800 **Manstein, D. J., Geeves, M. A. and Soldati, D.** (2002). Toxoplasma gondii myosin A and its light chain:
801 a fast, single-headed, plus-end-directed motor. *EMBO J,* **21**, 2149-2158. doi:
802 10.1093/emboj/21.9.2149.

803 **Hermosilla, C., Barbisch, B., Heise, A., Kowalik, S. and Zahner, H.** (2002). Development of Eimeria
804 bovis in vitro: suitability of several bovine, human and porcine endothelial cell lines, bovine fetal
805 gastrointestinal, Madin-Darby bovine kidney (MDBK) and African green monkey kidney (VERO) cells.
806 *Parasitol Res,* **88**, 301-307.

807 **Hu, K., Johnson, J., Florens, L., Fraunholz, M., Suravajjala, S., DiLullo, C., Yates, J., Roos, D. S. and**
808 **Murray, J. M.** (2006). Cytoskeletal components of an invasion machine--the apical complex of
809 Toxoplasma gondii. *PLoS Pathog,* **2**, e13. doi: 10.1371/journal.ppat.0020013.

810 **Hu, K., Roos, D. S. and Murray, J. M.** (2002). A novel polymer of tubulin forms the conoid of
811 Toxoplasma gondii. *J Cell Biol,* **156**, 1039-1050. doi: 10.1083/jcb.200112086.

812 **Itagaki, K., Hirayama, N., Tsubokura, M., Otsuki, K. and Taira, Y.** (1974). Devolopment of Eimeria
813 tenella, E. brunetti and E. acervulina in cell cultures. *Nihon Juigaku Zasshi,* **36**, 467-482.

814 **Jackson, A. J., Clucas, C., Mamczur, N. J., Ferguson, D. J. and Meissner, M.** (2013). Toxoplasma gondii
815 Syntaxin 6 is required for vesicular transport between endosomal-like compartments and the Golgi
816 complex. *Traffic,* **14**, 1166-1181. doi: 10.1111/tra.12102.

Jacot, D., Tosetti, N., Pires, I., Stock, J., Graindorge, A., Hung, Y. F., Han, H., Tewari, R., Kursula, I. and Soldati-Favre, D. (2016). An Apicomplexan Actin-Binding Protein Serves as a Connector and Lipid Sensor to Coordinate Motility and Invasion. *Cell Host Microbe,* **20**, 731-743. doi: 10.1016/j.chom.2016.10.020.

Jatau, I. D., Lawal, I. A., Kwaga, J. K., Tomley, F. M., Blake, D. P. and Nok, A. J. (2016). Three operational taxonomic units of Eimeria are common in Nigerian chickens and may undermine effective molecular diagnosis of coccidiosis. *BMC Vet Res,* **12**, 86. doi: 10.1186/s12917-016-0713-9.

Jensen, J. B. (1975). Ultrastructure of the invasion of Eimeria magna sporozoites into cultured cells. *J Protozool,* **22**, 411-415.

Jensen, J. B. and Edgar, S. A. (1976). Possible secretory function of the rhoptries of Eimeria magna during penetration of cultured cells. *J Parasitol,* **62**, 988-992.

Jeurissen, S. H., Janse, E. M., Vermeulen, A. N. and Vervelde, L. (1996). Eimeria tenella infections in chickens: aspects of host-parasite: interaction. *Vet Immunol Immunopathol,* **54**, 231-238.

Jirku, M., Modry, D., Slapeta, J. R., Koudela, B. and Lukes, J. (2002). The phylogeny of Goussia and Choleoeimeria (Apicomplexa; Eimeriorina) and the evolution of excystation structures in coccidia. *Protist,* **153**, 379-390. doi: 10.1078/14344610260450118.

Karkhanis, Y. D., Nollstadt, K. A., Bhogal, B. S., Ravino, O., Pellegrino, R., Crane, M. S., Murray, P. K. and Turner, M. J. (1991). Purification and characterization of a protective antigen from Eimeria tenella. *Infect Immun,* **59**, 983-989.

Kawazoe, U., Tomley, F. M. and Frazier, J. A. (1992). Fractionation and antigenic characterization of organelles of Eimeria tenella sporozoites. *Parasitology,* **104 Pt 1**, 1-9.

838 **Keeley, A. and Soldati, D.** (2004). The glideosome: a molecular machine powering motility and host-
839 cell invasion by Apicomplexa. *Trends Cell Biol,* **14**, 528-532. doi: 10.1016/j.tcb.2004.08.002.

840 **Kemp, L. E., Yamamoto, M. and Soldati-Favre, D.** (2013). Subversion of host cellular functions by the
841 apicomplexan parasites. *FEMS Microbiol Rev,* **37**, 607-631. doi: 10.1111/1574-6976.12013.

842 **King, C. A.** (1981). Cell surface interaction of the protozoan Gregarina with concanavalin A beads -
843 implications for models of gregarine gliding. *Cell Biol Int Rep,* **5**, 297-305.

844 **Koshy, A. A., Dietrich, H. K., Christian, D. A., Melehani, J. H., Shastri, A. J., Hunter, C. A. and**
845 **Boothroyd, J. C.** (2012). Toxoplasma co-opts host cells it does not invade. *PLoS Pathog,* **8**, e1002825.
846 doi: 10.1371/journal.ppat.1002825.

847 **Kremer, K., Kamin, D., Rittweger, E., Wilkes, J., Flammer, H., Mahler, S., Heng, J., Tonkin, C. J.,**
848 **Langsley, G., Hell, S. W., Carruthers, V. B., Ferguson, D. J. and Meissner, M.** (2013). An overexpression
849 screen of Toxoplasma gondii Rab-GTPases reveals distinct transport routes to the micronemes. *PLoS*
850 *Pathog,* **9**, e1003213. doi: 10.1371/journal.ppat.1003213.

851 **Kudryashev, M., Lepper, S., Stanway, R., Bohn, S., Baumeister, W., Cyrklaff, M. and Frischknecht, F.**
852 (2010). Positioning of large organelles by a membrane- associated cytoskeleton in Plasmodium
853 sporozoites. *Cell Microbiol,* **12**, 362-371. doi: 10.1111/j.1462-5822.2009.01399.x.

854 **LaFon, S. W. and Nelson, D. J.** (1985). Purine metabolism in the intact sporozoites and merozoites of
855 Eimeria tenella. *Mol Biochem Parasitol,* **14**, 11-22.

856 **Lai, L., Bumstead, J., Liu, Y., Garnett, J., Campanero-Rhodes, M. A., Blake, D. P., Palma, A. S., Chai,**
857 **W., Ferguson, D. J., Simpson, P., Feizi, T., Tomley, F. M. and Matthews, S.** (2011). The role of sialyl
858 glycan recognition in host tissue tropism of the avian parasite Eimeria tenella. *PLoS Pathog,* **7**,
859 e1002296. doi: 10.1371/journal.ppat.1002296.

Lai, L., Simpson, P., Bumstead, J., Tomley, F. and Matthews, S. (2009). Complete NMR assignments for the second microneme adhesive repeat (MAR) domain from Eimeria tenella microneme protein EtMIC3. *Biomol NMR Assign,* **3**, 175-177. doi: 10.1007/s12104-009-9168-2.

Lamarque, M., Besteiro, S., Papoin, J., Roques, M., Vulliez-Le Normand, B., Morlon-Guyot, J., Dubremetz, J. F., Fauquenoy, S., Tomavo, S., Faber, B. W., Kocken, C. H., Thomas, A. W., Boulanger, M. J., Bentley, G. A. and Lebrun, M. (2011). The RON2-AMA1 interaction is a critical step in moving junction-dependent invasion by apicomplexan parasites. *PLoS Pathog,* **7**, e1001276. doi: 10.1371/journal.ppat.1001276.

Lane, C. E. and Archibald, J. M. (2008). The eukaryotic tree of life: endosymbiosis takes its TOL. *Trends Ecol Evol,* **23**, 268-275. doi: 10.1016/j.tree.2008.02.004.

Lang, M., Kann, M., Zahner, H., Taubert, A. and Hermosilla, C. (2009). Inhibition of host cell apoptosis by Eimeria bovis sporozoites. *Vet Parasitol,* **160**, 25-33. doi: 10.1016/j.vetpar.2008.10.100.

Laurent, F., Bourdieu, C., Kaga, M., Chilmonczyk, S., Zgrzebski, G., Yvore, P. and Pery, P. (1993). Cloning and characterization of an Eimeria acervulina sporozoite gene homologous to aspartyl proteinases. *Mol Biochem Parasitol,* **62**, 303-312.

Lawn, A. M. and Rose, M. E. (1982). Mucosal transport of Eimeria tenella in the cecum of the chicken. *J Parasitol,* **68**, 1117-1123.

Lee, D. L. and Long, P. L. (1972). An electron microscopal study of Eimeria tenella grown in the liver of the chick embryo. *Int J Parasitol,* **2**, 55-58.

Leuckart, R. (1879). Die Parasiten des Menschen und die von ihnen herführenden Krankheiten, 2nd Edition. Winter, Leipzig und Heidelberg.

881 **Levine, N.D.** (1984). Nomenclatural corrections and new taxa in the apicomplexan protozoa.
882 *Transactions of the American Microscopical Society*, 195-204.

883 **Levine, P.** (1938). *Eimeria haganin*. sp. (Protozoa: Eimeriidae) a new coccidium of the chicken. *Cornell*
884 *Vet*, **28**, 263-266.

885 **Liberator, P. A., Hsu, J. and Turner, M. J.** (1989). Tandem trinucleotide repeats throughout the
886 nucleotide sequence of a cDNA encoding an Eimeria tenella sporozoite antigen. *Nucleic Acids Res,* **17**,
887 7104. doi: 10.1093/nar/17.17.7104.

888 **Lillehoj, H. S. and Trout, J. M.** (1993). Coccidia: a review of recent advances on immunity and vaccine
889 development. *Avian Pathol,* **22**, 3-31. doi: 10.1080/03079459308418897.

890 **Liu, S., Wang, L., Zheng, H., Xu, Z., Roellig, D. M., Li, N., Frace, M. A., Tang, K., Arrowood, M. J., Moss,**
891 **D. M., Zhang, L., Feng, Y. and Xiao, L.** (2016). Comparative genomics reveals Cyclospora cayetanensis
892 possesses coccidia-like metabolism and invasion components but unique surface antigens. *BMC*
893 *Genomics,* **17**, 316. doi: 10.1186/s12864-016-2632-3.

894 **Long, S., Anthony, B., Drewry, L. L. and Sibley, L. D.** (2017a). A conserved ankyrin repeat-containing
895 protein regulates conoid stability, motility and cell invasion in Toxoplasma gondii. *Nat Commun,* **8**,
896 2236. doi: 10.1038/s41467-017-02341-2.

897 **Long, S., Brown, K. M., Drewry, L. L., Anthony, B., Phan, I. Q. H. and Sibley, L. D.** (2017b). Calmodulin-
898 like proteins localized to the conoid regulate motility and cell invasion by Toxoplasma gondii. *PLoS*
899 *Pathog,* **13**, e1006379. doi: 10.1371/journal.ppat.1006379.

900 **Lourido, S., Shuman, J., Zhang, C., Shokat, K. M., Hui, R. and Sibley, L. D.** (2010). Calcium-dependent
901 protein kinase 1 is an essential regulator of exocytosis in Toxoplasma. *Nature,* **465**, 359-362. doi:
902 10.1038/nature09022.

Lourido, S., Tang, K. and Sibley, L. D. (2012). Distinct signalling pathways control Toxoplasma egress and host-cell invasion. *EMBO J,* **31**, 4524-4534. doi: 10.1038/emboj.2012.299.

Lutz, K., Schmitt, S., Linder, M., Hermosilla, C., Zahner, H. and Taubert, A. (2011). Eimeria bovis-induced modulation of the host cell proteome at the meront I stage. *Mol Biochem Parasitol,* **175**, 1-9. doi: 10.1016/j.molbiopara.2010.08.003.

Madden, P. A. and Vetterling, J. M. (1977). Scanning electron microscopy of Eimeria tenella microgametogenesis and fertilization. *J Parasitol,* **63**, 607-610.

Mai, K., Sharman, P. A., Walker, R. A., Katrib, M., De Souza, D., McConville, M. J., Wallach, M. G., Belli, S. I., Ferguson, D. J. and Smith, N. C. (2009). Oocyst wall formation and composition in coccidian parasites. *Mem Inst Oswaldo Cruz,* **104**, 281-289. doi: 10.1590/s0074-02762009000200022.

McDonald, V. and Rose, M. E. (1987). Eimeria tenella and E. necatrix: a third generation of schizogony is an obligatory part of the developmental cycle. *J Parasitol,* **73**, 617-622.

McGovern, O. L. and Carruthers, V. B. (2016). Toxoplasma Retromer Is Here to Stay. *Trends Parasitol,* **32**, 758-760. doi: 10.1016/j.pt.2016.05.007.

McGovern, O. L., Rivera-Cuevas, Y., Kannan, G., Narwold, A. J., Jr. and Carruthers, V. B. (2018). Intersection of endocytic and exocytic systems in Toxoplasma gondii. *Traffic,* **19**, 336-353. doi: 10.1111/tra.12556.

McLaren, D. J. (1969). Observations on the fine structural changes associated with schizogony and gametogony in Eimeria tenella. *Parasitology,* **59**, 563-574.

McLaren, D. J. and Paget, G. E. (1968). A fine structural study on the merozoite of Eimeria tenella with special reference to the conoid apparatus. *Parasitology,* **58**, 561-571.

Megía-Palma, R., Martínez, J., Acevedo, I., Martín, J., García-Roa, R., Ortega, J., Peso-Fernández, M., Albaladejo, G., Cooper, R.D., Paranjpe, D.A. (2015). Phylogeny of the reptilian *Eimeria*: are Choleoeimeria and Acroeimeria valid generic names? *Zoologica Scripta*, **44**, 684-692.

Melo, E. J., Attias, M. and De Souza, W. (2000). The single mitochondrion of tachyzoites of Toxoplasma gondii. *J Struct Biol,* **130**, 27-33. doi: 10.1006/jsbi.2000.4228.

Mercier, C. and Cesbron-Delauw, M. F. (2015). Toxoplasma secretory granules: one population or more? *Trends Parasitol,* **31**, 60-71. doi: 10.1016/j.pt.2014.12.002.

Miller, G. A., Bhogal, B. S., McCandliss, R., Strausberg, R. L., Jessee, E. J., Anderson, A. C., Fuchs, C. K., Nagle, J., Likel, M. H., Strasser, J. M. and et al. (1989). Characterization and vaccine potential of a novel recombinant coccidial antigen. *Infect Immun,* **57**, 2014-2020.

Miller, L. H., McAuliffe, F. M. and Johnson, J. G. (1979). Invasion of erythrocytes by malaria merozoites. *Prog Clin Biol Res,* **30**, 497-502.

Miranda, K., de Souza, W., Plattner, H., Hentschel, J., Kawazoe, U., Fang, J. and Moreno, S. N. (2008). Acidocalcisomes in Apicomplexan parasites. *Exp Parasitol,* **118**, 2-9. doi: 10.1016/j.exppara.2007.07.009.

Mordue, D. G., Hakansson, S., Niesman, I. and Sibley, L. D. (1999). Toxoplasma gondii resides in a vacuole that avoids fusion with host cell endocytic and exocytic vesicular trafficking pathways. *Exp Parasitol,* **92**, 87-99.

Mordue, D. G. and Sibley, L. D. (1997). Intracellular fate of vacuoles containing Toxoplasma gondii is determined at the time of formation and depends on the mechanism of entry. *J Immunol,* **159**, 4452-4459.

Morlon-Guyot, J., El Hajj, H., Martin, K., Fois, A., Carrillo, A., Berry, L., Burchmore, R., Meissner, M., Lebrun, M. and Daher, W. (2018). A proteomic analysis unravels novel CORVET and HOPS proteins involved in Toxoplasma gondii secretory organelles biogenesis. *Cell Microbiol,* **20**, e12870. doi: 10.1111/cmi.12870.

Morlon-Guyot, J., Pastore, S., Berry, L., Lebrun, M. and Daher, W. (2015). Toxoplasma gondii Vps11, a subunit of HOPS and CORVET tethering complexes, is essential for the biogenesis of secretory organelles. *Cell Microbiol,* **17**, 1157-1178. doi: 10.1111/cmi.12426.

Morrissette, N. S. and Sibley, L. D. (2002). Cytoskeleton of apicomplexan parasites. *Microbiol Mol Biol Rev,* **66**, 21-38; table of contents. doi: 10.1128/mmbr.66.1.21-38.2002.

Mota, M. M., Pradel, G., Vanderberg, J. P., Hafalla, J. C., Frevert, U., Nussenzweig, R. S., Nussenzweig, V. and Rodriguez, A. (2001). Migration of Plasmodium sporozoites through cells before infection. *Science,* **291**, 141-144. doi: 10.1126/science.291.5501.141.

Mota, M. M. and Rodriguez, A. (2001). Migration through host cells by apicomplexan parasites. *Microbes Infect,* **3**, 1123-1128.

Nishi, M., Hu, K., Murray, J. M. and Roos, D. S. (2008). Organellar dynamics during the cell cycle of Toxoplasma gondii. *J Cell Sci,* **121**, 1559-1568. doi: 10.1242/jcs.021089.

Novilla, M. N. and Carpenter, J. W. (2004). Pathology and pathogenesis of disseminated visceral coccidiosis in cranes. *Avian Pathol,* **33**, 275-280. doi: 10.1080/0307945042000203371.

Oakes, R. D., Kurian, D., Bromley, E., Ward, C., Lal, K., Blake, D. P., Reid, A. J., Pain, A., Sinden, R. E., Wastling, J. M. and Tomley, F. M. (2013). The rhoptry proteome of Eimeria tenella sporozoites. *Int J Parasitol,* **43**, 181-188. doi: 10.1016/j.ijpara.2012.10.024.

966 **Opitz, C. and Soldati, D.** (2002). 'The glideosome': a dynamic complex powering gliding motion and
967 host cell invasion by Toxoplasma gondii. *Mol Microbiol,* **45**, 597-604. doi: 10.1046/j.1365-
968 2958.2002.03056.x.

969 **Pacheco, N. D., Vetterling, J. M. and Doran, D. J.** (1975). Ultrastructure of cytoplasmic and nuclear
970 changes in Eimeria tenella during first-generation schizogony in cell culture. *J Parasitol,* **61**, 31-42.

971 **Pakandl, M.** (2009). Coccidia of rabbit: a review. *Folia Parasitol (Praha),* **56**, 153-166.

972 **Pan, M., Zhou, Y., Wang, Y., Li, L., Song, Y., Hou, L. and Zhao, J.** (2017). Screening and Identification
973 of the Host Proteins Interacting with Toxoplasma gondii Rhoptry Protein ROP16. *Front Microbiol,* **8**,
974 2408. doi: 10.3389/fmicb.2017.02408.

975 **Paredes-Santos, T. C., de Souza, W. and Attias, M.** (2012). Dynamics and 3D organization of secretory
976 organelles of Toxoplasma gondii. *J Struct Biol,* **177**, 420-430. doi: 10.1016/j.jsb.2011.11.028.

977 **Pastor-Fernandez, I., Kim, S., Billington, K., Bumstead, J., Marugan-Hernandez, V., Kuster, T.,**
978 **Ferguson, D. J. P., Vervelde, L., Blake, D. P. and Tomley, F. M.** (2018). Development of cross-protective
979 Eimeria-vectored vaccines based on apical membrane antigens. *Int J Parasitol,* **48**, 505-518. doi:
980 10.1016/j.ijpara.2018.01.003.

981 **Peek, H. W. and Landman, W. J.** (2011). Coccidiosis in poultry: anticoccidial products, vaccines and
982 other prevention strategies. *Vet Q,* **31**, 143-161. doi: 10.1080/01652176.2011.605247.

983 **Pieperhoff, M. S., Schmitt, M., Ferguson, D. J. and Meissner, M.** (2013). The role of clathrin in post-
984 Golgi trafficking in Toxoplasma gondii. *PLoS One,* **8**, e77620. doi: 10.1371/journal.pone.0077620.

985 **Pu, Y. H. and Zhang, D. L.** (2012). [Research progress on invasion mechanism and immunology of
986 Toxoplasma gondii]. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi,* **30**, 480-485, 490.

Reid, A. J., Blake, D. P., Ansari, H. R., Billington, K., Browne, H. P., Bryant, J., Dunn, M., Hung, S. S., Kawahara, F., Miranda-Saavedra, D., Malas, T. B., Mourier, T., Naghra, H., Nair, M., Otto, T. D., Rawlings, N. D., Rivailler, P., Sanchez-Flores, A., Sanders, M., Subramaniam, C., Tay, Y. L., Woo, Y., Wu, X., Barrell, B., Dear, P. H., Doerig, C., Gruber, A., Ivens, A. C., Parkinson, J., Rajandream, M. A., Shirley, M. W., Wan, K. L., Berriman, M., Tomley, F. M. and Pain, A. (2014). Genomic analysis of the causative agents of coccidiosis in domestic chickens. *Genome Res,* **24**, 1676-1685. doi: 10.1101/gr.168955.113.

Relman, D. A., Schmidt, T. M., Gajadhar, A., Sogin, M., Cross, J., Yoder, K., Sethabutr, O. and Echeverria, P. (1996). Molecular phylogenetic analysis of Cyclospora, the human intestinal pathogen, suggests that it is closely related to Eimeria species. *J Infect Dis,* **173**, 440-445. doi: 10.1093/infdis/173.2.440.

Roberts, W. L. and Hammond, D. M. (1970). Ultrastructural and cytologic studies of the sporozoites of four Eimeria species. *J Protozool,* **17**, 76-86.

Roberts, W. L., Hammond, D. M. and Speer, C. A. (1970a). Ultrastructural study of the intra- and extracellular sporozoites of Eimeria callospermophili. *J Parasitol,* **56**, 907-917.

Roberts, W. L., Speer, C. A. and Hammond, D. M. (1970b). Electron and light microscope studies of the oocyst walls, sporocysts, and excysting sporozoites of Eimeria callospermophili and E. larimerensis. *J Parasitol,* **56**, 918-926.

Roberts, W. L., Speer, C. A. and Hammond, D. M. (1971). Penetration of Eimeria larimerensis sporozoites into cultured cells as observed with the light and electron microscopes. *J Parasitol,* **57**, 615-625.

Roiko, M. S. and Carruthers, V. B. (2013). Functional dissection of Toxoplasma gondii perforin-like protein 1 reveals a dual domain mode of membrane binding for cytolysis and parasite egress. *J Biol Chem,* **288**, 8712-8725. doi: 10.1074/jbc.M113.450932.

Russell, D. G. and Sinden, R. E. (1981). The role of the cytoskeleton in the motility of coccidian sporozoites. *J Cell Sci,* **50**, 345-359.

Russell, D. G. and Sinden, R. E. (1982). Three-dimensional study of the intact cytoskeleton of coccidian sporozoites. *Int J Parasitol,* **12**, 221-226.

Ryan, R., Shirley, M. and Tomley, F. (2000). Mapping and expression of microneme genes in Eimeria tenella. *Int J Parasitol,* **30**, 1493-1499.

Ryley, J. F. (1969). Ultrastructural studies on the sporozoite of Eimeria tenella. *Parasitology,* **59**, 67-72.

Ryley, J. F., Bentley, M., Manners, D. J. and Stark, J. R. (1969). Amylopectin, the storage polysaccharide of the Coccidia Eimeria brunetti and E. tenella. *J Parasitol,* **55**, 839-845.

Sakura, T., Sindikubwabo, F., Oesterlin, L. K., Bousquet, H., Slomianny, C., Hakimi, M. A., Langsley, G. and Tomavo, S. (2016). A Critical Role for Toxoplasma gondii Vacuolar Protein Sorting VPS9 in Secretory Organelle Biogenesis and Host Infection. *Sci Rep,* **6**, 38842. doi: 10.1038/srep38842.

Sangare, L. O., Alayi, T. D., Westermann, B., Hovasse, A., Sindikubwabo, F., Callebaut, I., Werkmeister, E., Lafont, F., Slomianny, C., Hakimi, M. A., Van Dorsselaer, A., Schaeffer-Reiss, C. and Tomavo, S. (2016). Unconventional endosome-like compartment and retromer complex in Toxoplasma gondii govern parasite integrity and host infection. *Nat Commun,* **7**, 11191. doi: 10.1038/ncomms11191.

Schneider, A. (1875). Note sur la psorospermies oviformes du poulpe. *Archives de Zoologie Experimentale et Generale*, 387–404.

Scholtyseck, E. (1965). Elektronenmikroskopische Untersuchungen über die Schizogonie bei Coccidien (*Eimeria perforans* und *E. stiedae*). *Parasitology Research*, **26**, 50-62.

Sheffield, H. G. and Hammond, D. M. (1966). Fine structure of first-generation merozoites of Eimeria bovis. *J Parasitol,* **52**, 595-606.

Shirley, M. W., Smith, A. L. and Blake, D. P. (2007). Challenges in the successful control of the avian coccidia. *Vaccine,* **25**, 5540-5547.

Shirley, M. W., Smith, A. L. and Tomley, F. M. (2005). The biology of avian Eimeria with an emphasis on their control by vaccination. *Adv Parasitol,* **60**, 285-330.

Sibley, L. D. (2010). How apicomplexan parasites move in and out of cells. *Curr Opin Biotechnol,* **21**, 592-598. doi: 10.1016/j.copbio.2010.05.009.

Sinden, R. E. and Croll, N. A. (1975). Cytology and kinetics of microgametogenesis and fertilization in Plasmodium yoelii nigeriensis. *Parasitology,* **70**, 53-65.

Sloves, P. J., Delhaye, S., Mouveaux, T., Werkmeister, E., Slomianny, C., Hovasse, A., Dilezitoko Alayi, T., Callebaut, I., Gaji, R. Y., Schaeffer-Reiss, C., Van Dorsselear, A., Carruthers, V. B. and Tomavo, S. (2012). Toxoplasma sortilin-like receptor regulates protein transport and is essential for apical secretory organelle biogenesis and host infection. *Cell Host Microbe,* **11**, 515-527. doi: 10.1016/j.chom.2012.03.006.

Soares Medeiros, L. C., Gomes, F., Maciel, L. R., Seabra, S. H., Docampo, R., Moreno, S., Plattner, H., Hentschel, J., Kawazoe, U., Barrabin, H., de Souza, W., Damatta, R. A. and Miranda, K. (2011). Volutin granules of Eimeria parasites are acidic compartments and have physiological and structural

1051 characteristics similar to acidocalcisomes. *J Eukaryot Microbiol,* **58**, 416-423. doi: 10.1111/j.1550-
1052 7408.2011.00565.x.

1053 **Song, X., Xu, L., Yan, R., Huang, X. and Li, X.** (2015). Construction of Eimeria tenella multi-epitope DNA
1054 vaccines and their protective efficacies against experimental infection. *Vet Immunol Immunopathol,*
1055 **166**, 79-87. doi: 10.1016/j.vetimm.2015.05.005.

1056 **Stockdale, P. H. and Fernando, M. A.** (1975). The development of the lesions caused by second
1057 generation schizonts of Eimeria necatrix. *Res Vet Sci,* **19**, 204-208.

1058 **Stotish, R. L., Wang, C. C. and Meyenhofer, M.** (1978). Structure and composition of the oocyst wall
1059 of Eimeria tenella. *J Parasitol,* **64**, 1074-1081.

1060 **Striepen, B., Crawford, M. J., Shaw, M. K., Tilney, L. G., Seeber, F. and Roos, D. S.** (2000). The plastid
1061 of Toxoplasma gondii is divided by association with the centrosomes. *J Cell Biol,* **151**, 1423-1434. doi:
1062 10.1083/jcb.151.7.1423.

1063 **Strout, R. G. and Scholtyseck, E.** (1970). The ultrastructure of first generation development of Eimeria
1064 tenella (Railliet and Lucet, 1891) Fantham, 1909 in cell cultures. *Z Parasitenkd,* **35**, 87-96.

1065 **Suss-Toby, E., Zimmerberg, J. and Ward, G. E.** (1996). Toxoplasma invasion: the parasitophorous
1066 vacuole is formed from host cell plasma membrane and pinches off via a fission pore. *Proc Natl Acad*
1067 *Sci U S A,* **93**, 8413-8418. doi: 10.1073/pnas.93.16.8413.

1068 **Talevich, E. and Kannan, N.** (2013). Structural and evolutionary adaptation of rhoptry kinases and
1069 pseudokinases, a family of coccidian virulence factors. *BMC Evol Biol,* **13**, 117. doi: 10.1186/1471-
1070 2148-13-117.

Tenter, A. M., Barta, J. R., Beveridge, I., Duszynski, D. W., Mehlhorn, H., Morrison, D. A., Thompson, R. C. and Conrad, P. A. (2002). The conceptual basis for a new classification of the coccidia. *Int J Parasitol,* **32**, 595-616.

Tenter, A. M. and Johnson, A. M. (1997). Phylogeny of the tissue cyst-forming coccidia. *Adv Parasitol,* **39**, 69-139.

Tomavo, S., Slomianny, C., Meissner, M. and Carruthers, V. B. (2013). Protein trafficking through the endosomal system prepares intracellular parasites for a home invasion. *PLoS Pathog,* **9**, e1003629. doi: 10.1371/journal.ppat.1003629.

Tomley, F. M. (1994). Characterization of rhoptry proteins of Eimeria tenella sporozoites: antigenic diversity of rhoptry epitopes within species of the genus Eimeria and among three asexual generations of a single species, E. tenella. *Infect Immun,* **62**, 4656-4658.

Tomley, F. M., Bumstead, J. M., Billington, K. J. and Dunn, P. P. (1996). Molecular cloning and characterization of a novel acidic microneme protein (Etmic-2) from the apicomplexan protozoan parasite, Eimeria tenella. *Mol Biochem Parasitol,* **79**, 195-206.

Tomley, F. M. and Soldati, D. S. (2001). Mix and match modules: structure and function of microneme proteins in apicomplexan parasites. *Trends Parasitol,* **17**, 81-88.

Trees, A.J. (2001). Parasitic diseases. In: F.Jordan, M.Pattison, D.Alexander and T.Faragher (eds). Poultry Diseases, 5th edition. Vol Poultry diseases. 5th Edition. W.B.Saunders Ltd., 405-433 pp.

Tyler, J. S. and Boothroyd, J. C. (2011). The C-terminus of Toxoplasma RON2 provides the crucial link between AMA1 and the host-associated invasion complex. *PLoS Pathog,* **7**, e1001282. doi: 10.1371/journal.ppat.1001282.

Vanderberg, J. P. (1974). Studies on the motility of Plasmodium sporozoites. *J Protozool,* **21**, 527-537.

Venugopal, K., Werkmeister, E., Barois, N., Saliou, J. M., Poncet, A., Huot, L., Sindikubwabo, F., Hakimi, M. A., Langsley, G., Lafont, F. and Marion, S. (2017). Dual role of the Toxoplasma gondii clathrin adaptor AP1 in the sorting of rhoptry and microneme proteins and in parasite division. *PLoS Pathog,* **13**, e1006331. doi: 10.1371/journal.ppat.1006331.

Vermeulen, A. N., Kok, J. J., van den Boogaart, P., Dijkema, R. and Claessens, J. A. (1993). Eimeria refractile body proteins contain two potentially functional characteristics: transhydrogenase and carbohydrate transport. *FEMS Microbiol Lett,* **110**, 223-229. doi: 10.1111/j.1574-6968.1993.tb06324.x.

Vetterling, J. M., Pacheco, N. D. and Madden, P. A. (1973). Ultrastructure of dormant, activated, and intracellular sporozoites of Eimeria adenoeides and E. tenella. *J Parasitol,* **59**, 15-27.

Walker, R. A., Ferguson, D. J., Miller, C. M. and Smith, N. C. (2013). Sex and Eimeria: a molecular perspective. *Parasitology,* **140**, 1701-1717. doi: 10.1017/S0031182013000838.

Walker, R. A., Sharman, P. A., Miller, C. M., Lippuner, C., Okoniewski, M., Eichenberger, R. M., Ramakrishnan, C., Brossier, F., Deplazes, P., Hehl, A. B. and Smith, N. C. (2015). RNA Seq analysis of the Eimeria tenella gametocyte transcriptome reveals clues about the molecular basis for sexual reproduction and oocyst biogenesis. *BMC Genomics,* **16**, 94. doi: 10.1186/s12864-015-1298-6.

West, S. A., Reece, S. E. and Read, A. F. (2003). Toxoplasma gondii, sex and premature rejection. *Trends Parasitol,* **19**, 155-157; discussion 157-158.

Wiersma, H. I., Galuska, S. E., Tomley, F. M., Sibley, L. D., Liberator, P. A. and Donald, R. G. (2004). A role for coccidian cGMP-dependent protein kinase in motility and invasion. *Int J Parasitol,* **34**, 369-380. doi: 10.1016/j.ijpara.2003.11.019.

Wohlfert, E. A., Blader, I. J. and Wilson, E. H. (2017). Brains and Brawn: Toxoplasma Infections of the Central Nervous System and Skeletal Muscle. *Trends Parasitol,* **33**, 519-531. doi: 10.1016/j.pt.2017.04.001.

Yan, X., Liu, X., Ji, Y., Tao, G. and Suo, X. (2015). Ethanol and isopropanol trigger rapid egress of intracellular Eimeria tenella sporozoites. *Parasitol Res,* **114**, 625-630. doi: 10.1007/s00436-014-4224-y.

Yan, X., Tao, G., Liu, X., Ji, Y. and Suo, X. (2016). Calcium-dependent microneme protein discharge and in vitro egress of Eimeria tenella sporozoites. *Exp Parasitol,* **170**, 193-197. doi: 10.1016/j.exppara.2016.09.005.

Yang, G., Yao, J., Yang, W., Jiang, Y., Du, J., Huang, H., Gu, W., Hu, J., Ye, L., Shi, C., Shan, B. and Wang, C. (2017). Construction and immunological evaluation of recombinant Lactobacillus plantarum expressing SO7 of Eimeria tenella fusion DC-targeting peptide. *Vet Parasitol,* **236**, 7-13. doi: 10.1016/j.vetpar.2017.01.023.

Yun, C. H., Lillehoj, H. S. and Choi, K. D. (2000a). Eimeria tenella infection induces local gamma interferon production and intestinal lymphocyte subpopulation changes. *Infect Immun,* **68**, 1282-1288.

Yun, C. H., Lillehoj, H. S. and Lillehoj, E. P. (2000b). Intestinal immune responses to coccidiosis. *Dev Comp Immunol,* **24**, 303-324.

1132 TABLES

1133 Table 1. Comparison of the seven known *Eimeria* species affecting chickens in terms of site of
1134 infection, level of pathology and oocyst morphology.

1135 FIGURE LEGENDS

1136 Figure 1. Phylogenetic tree showing the relationships between relevant apicomplexan species. The
1137 tree was generated based on data from the following sources: (Adl *et al.*, 2007; Carreno *et al.*, 1999;
1138 Golemansky, 2015; Jirku *et al.*, 2002; Lane & Archibald, 2008; Levine, 1984; Megia-Palma *et al*., 2015;
1139 Tenter *et al.*, 2002; Tenter & Johnson, 1997).

1140 Figure 2. Life cycle of *Eimeria tenella*. Numbers correlate with subsequent stages of the development:
1141 1. Oocyst sporulation in the environment and oral ingestion by the chicken; 2. Release of sporocysts
1142 and sporozoites along the transit in the chicken digestive system; 3. Active invasion of sporozoites in
1143 the ceaeca epithelium and formation of the intracellular trophozoite within the parasitophorous
1144 vacuole; 4. First round of shyzogony and release of first generation merozoites; 5. Second and third
1145 round of shyzogony and release of second and third generation merozoites, respectively; 6.
1146 Development of microgametes and macrogametes (gametogony) and fecundation; 7. Zygote,
1147 development of the oocyst and release to the environment as unsporulated oocyst.

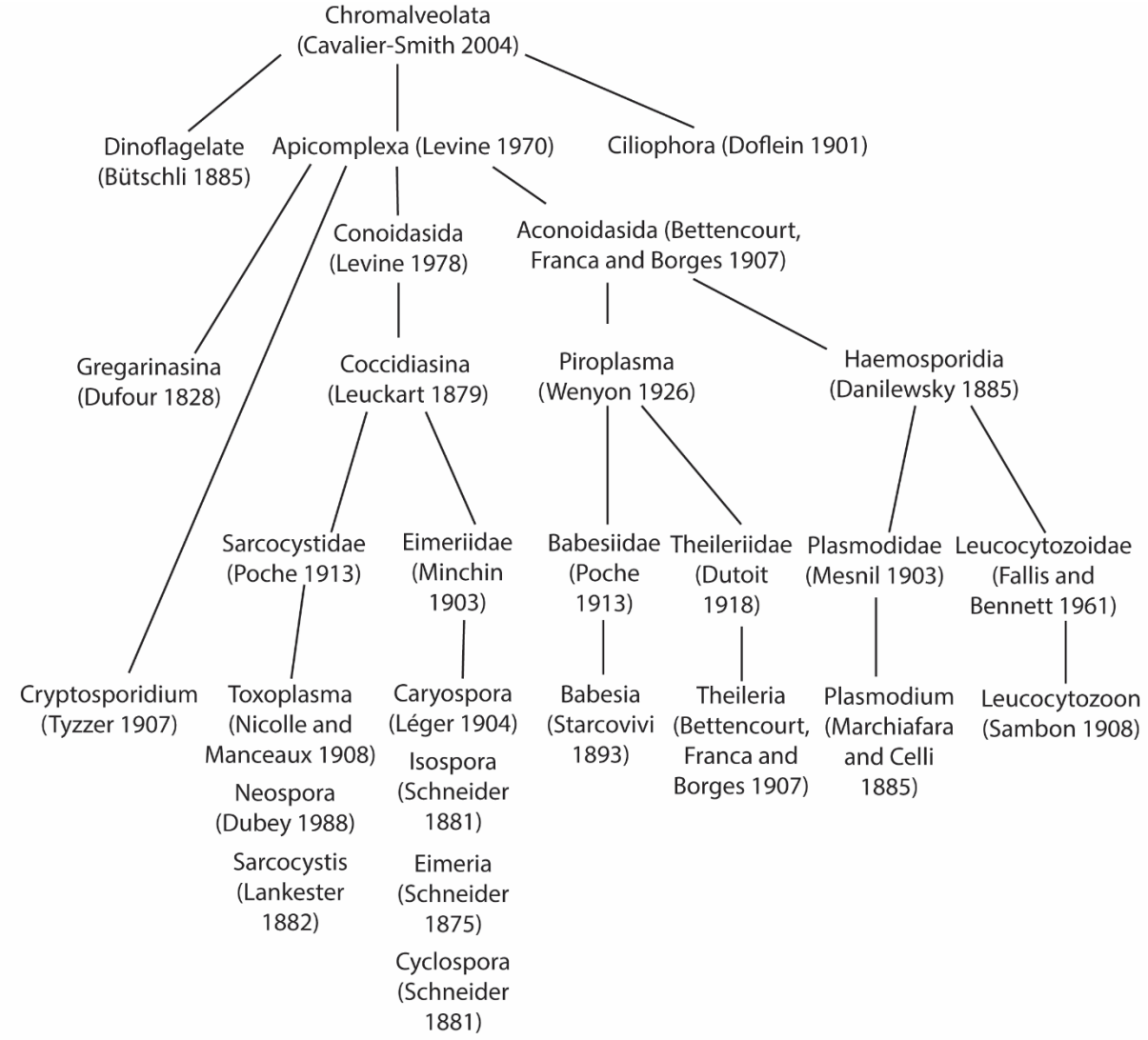
1148 Figure 3. Sporulated oocysts of Eimeria tenella. The oocysts contains four sporocysts (arrowheads),
1149 each containing two sporozoites (asterisks) and a micropyle at each sporocysts apex (arrow).

1150 Figure 4. Transmission electron micrographs of *Eimeria tenella* sporozoite ultrastructures (A. Burrell,
1151 unpublished). A. Sporozoites with two large non-membrane bound organelles known as anterior and
1152 posterior refractile bodies (ARB and PRB) situated at either side of the nucleus (N) as well as numerous
1153 amylopectin granules (arrowhead). B. Micronemes and rhoptries (asterisks) occupying most of the
1154 cytoplasm in the anterior quarter of the cell. C. Apex of the cell with a cone shaped structures

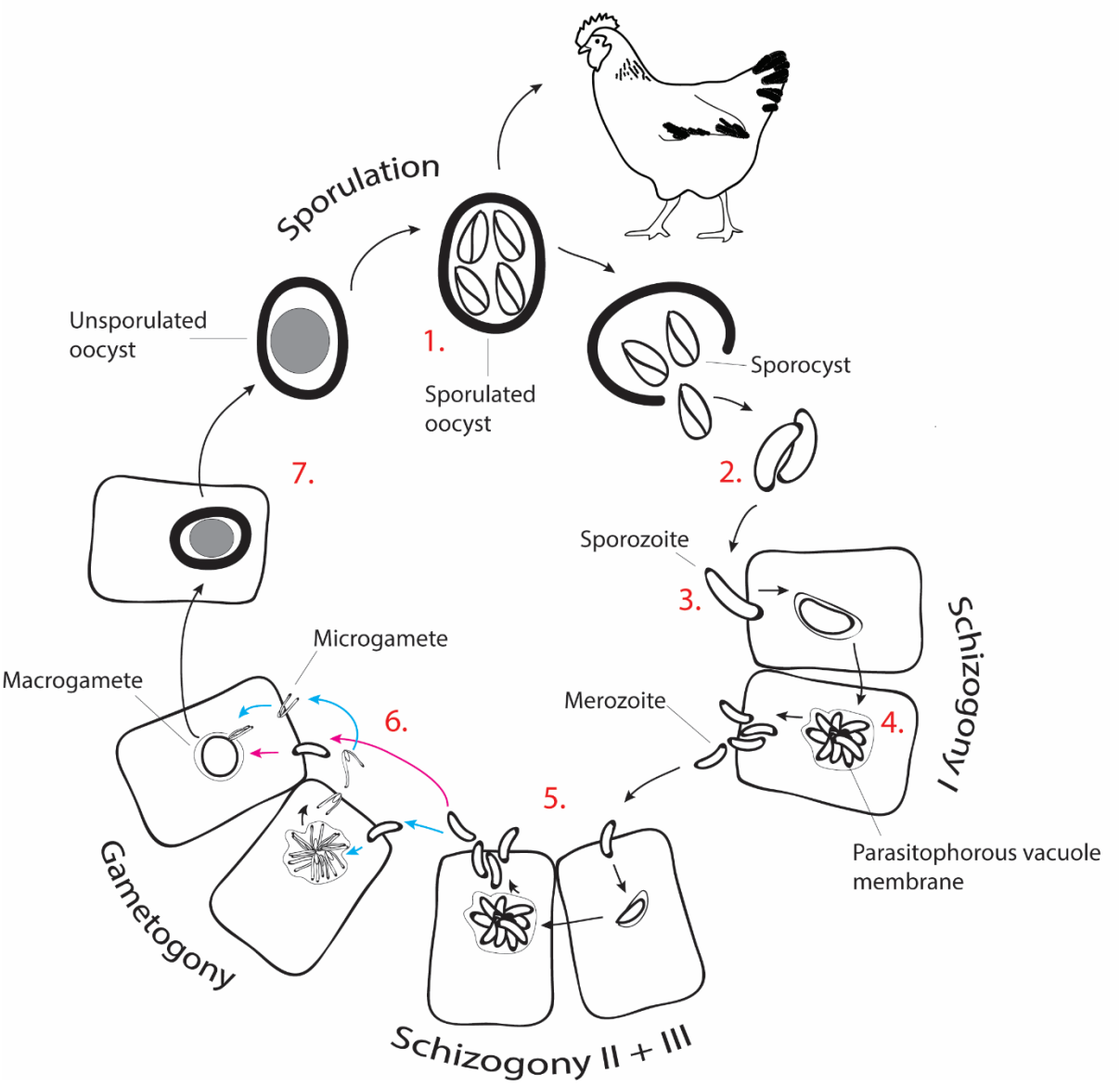
composed of helical fibers known as the conoid (arrow) sitting within the apical polar rings (arrowheads). D. Centrally located nucleus (N) next to which the Golgi apparatus can be observed (arrowhead). E. Mitochondrion cross-section showing plump cristae. F. Triple-layered pellicle (arrowheads) consisting of plasma membrane and inner membrane complex beneath which sits an array of sub-pellicular microtubules (arrows). G. Apicoplast with four membrane layers. Scale bars: A ~2µm; B-G ~500nm.

Figure 5. Cell structure and organelle content of different coccidian cells. A. Tachyzoite of *Toxoplasma gondii*. B. Sporozoite of *Eimeria* species. C. Merozoite of *Eimeria* species.

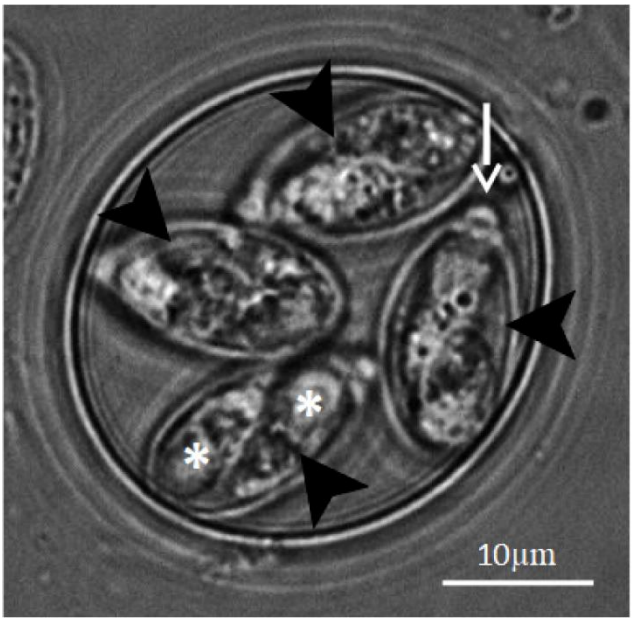
Figure 6. Schyzogony (adapted from Francia and Striepen, 2014). 1. Trophozoite development after sporozoite invasion 2. Immature schyzont, nuclei multiply by several rounds of mitosis. 3. Mature schyzont, the last round of division coincides with the merozoites budding at the parasite surface. Merozoites are release and initiate a new round of schyzogony (or gametogony).


Chromalveolata
(Cavalier-Smith 2004)
Dinoflagelate
(Bütschli 1885)
Apicomplexa (Levine 1970)
Ciliophora (Doflein 1901)
Gregarinasina
(Dufour 1828)
Conoidasida
(Levine 1978)
Aconoidasida (Bettencourt,
Franca and Borges 1907)
Coccidiasina
(Leuckart 1879)
Piroplasma
(Wenyon 1926)
Haemosporidia
(Danilewsky 1885)
Sarcocystidae
(Poche 1913)
Eimeriidae
(Minchin
1903)
Babesiidae
(Poche
1913)
Theileriidae
(Dutoit
1918)
Plasmodidae
(Mesnil 1903)
Leucocytozoidae
(Fallis and
Bennett 1961)
Cryptosporidium
(Tyzzer 1907)
Toxoplasma
(Nicolle and
Manceaux 1908)
Neospora
(Dubey 1988)
Sarcocystis
(Lankester
1882)
Caryospora
(Léger 1904)
Isospora
(Schneider
1881)
Eimeria
(Schneider
1875)
Cyclospora
(Schneider
1881)
Babesia
(Starcovivi
1893)
Theileria
(Bettencourt,
Franca and
Borges 1907)
Plasmodium
(Marchiafara
and Celli
1885)
Leucocytozoon
(Sambon 1908)

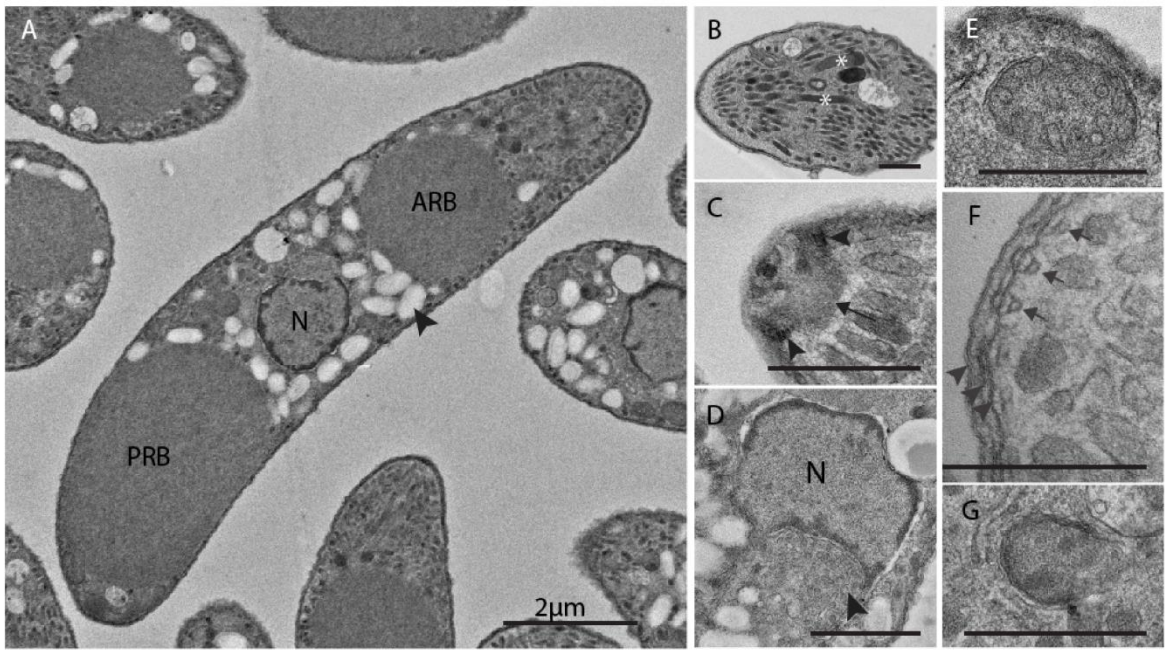
1167


Sporulation
Unsporulated oocyst
1.
Sporulated oocyst
Sporocyst
2.
Sporozoite
3.
Schizogony I
Merozoite
4.
Parasitophorous vacuole membrane
5.
Schizogony II + III
6.
Microgamete
Macrogamete
Gametogony
7.

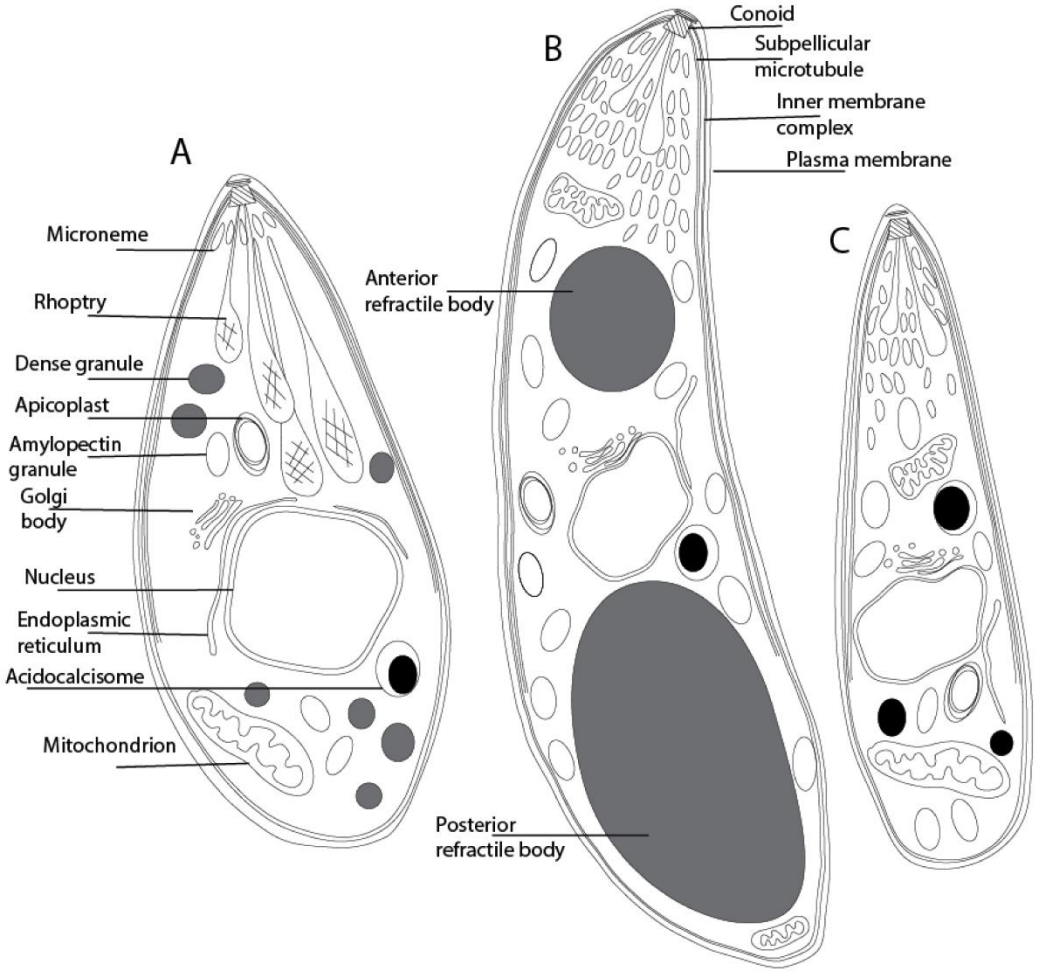
1168


10µm

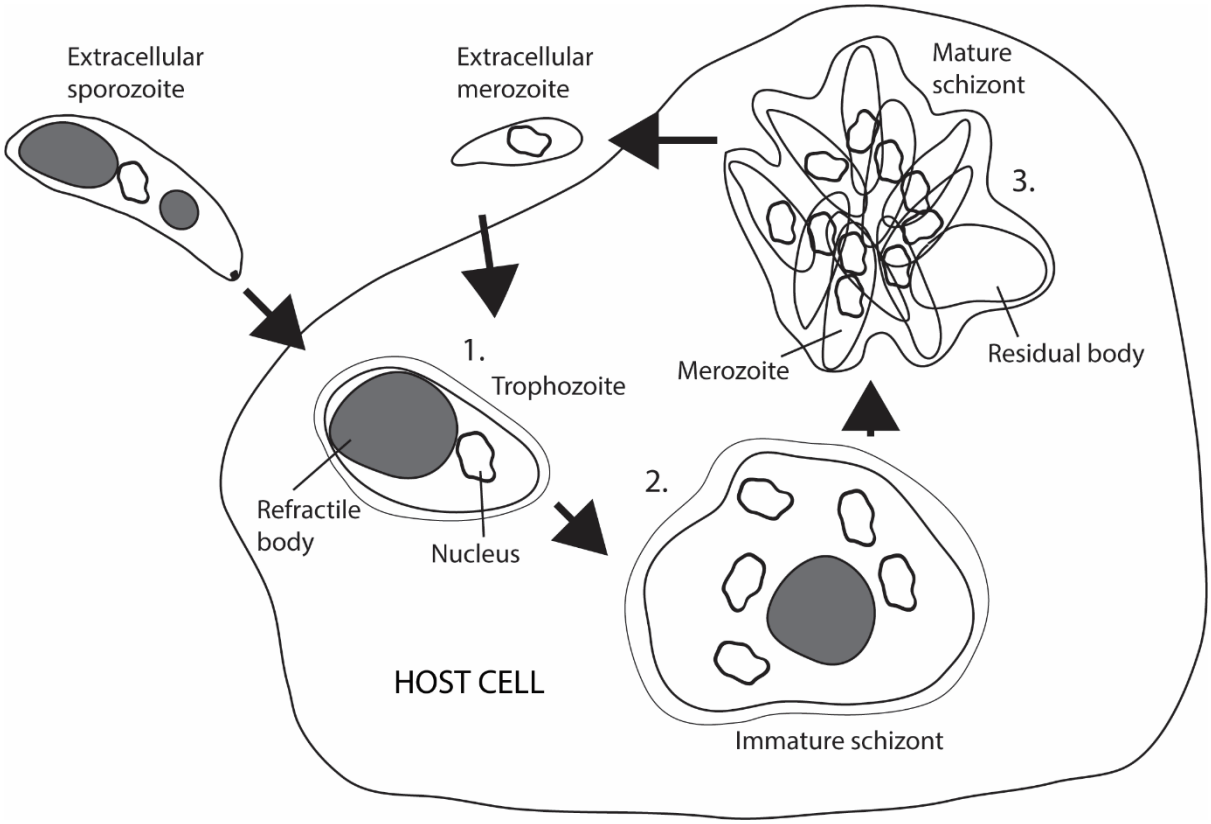
1169


A
ARB
N
PRB
2µm
B
C
D
N
E
F
G

1170


A
Microneme
Rhoptry
Dense granule
Apicoplast
Amylopectin granule
Golgi body
Nucleus
Endoplasmic reticulum
Acidocalcisome
Mitochondrion
B
Conoid
Subpellicular microtubule
Inner membrane complex
Plasma membrane
Anterior refractile body
Posterior refractile body
C

1171


Extracellular
sporozoite
Extracellular
merozoite
Mature
schizont
3.
1.
Trophozoite
Merozoite
Residual body
Refractile
body
Nucleus
2.
HOST CELL
Immature schizont

1172