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REVIEW

Ultrastructural and developmental affinities of piscine coccidia

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ABSTRACT: Piscine coccidia differ from terrestrial-host coccidia in having a soft, membranous oocyst wall. In contrast, however, to the structural and developmental conformity observed among the highly evolved and specialized monoxenous eimeriid coccidia of avian and mammalian hosts, piscine coccidia demonstrate extreme diversity in developmental sites, sporocyst morphology, macrogamont organization and oocyst wall formation. Sporozoites and some merozoites of several piscine coccidia contain refractile bodies, while those of others contain crystalline ones. Some are obligatorily heteroxenous, while in others transmission is mediated by paratenic hosts. Structural and developmental variability among piscine coccidia could imply a polyphyletic origin, but it could also be an evidence for a lower degree of evolutionary specialization. Many of the structural and developmental features found in piscine coccidia occur in other lower vertebrate and invertebrate-host coccidia, as well as in the heteroxenous cyst-forming coccidia of higher vertebrates.

KEY WORDS: Coccidia · Fishes · Ultrastructure Development · Transmission · Diversity · Phylogeny

INTRODUCTION

Piscine coccidia differ structurally and in the way they develop in their hosts from homeothermic vertebrate coccidians. They have a soft, membranous oocyst wall (Fig. 1) (Dyková & Lom 1981, Paperna & Cross 1985) and their sporocysts lack true Stieda and Substieda bodies (Paperna & Landsberg 1985, Davies 1990, Azevedo et al. 1993). Piscine coccidia demonstrate extreme morphological and biological diversity, and consequently have been divided into several genera (Dyková & Lom 1981, Overstreet et al. 1984, Davies & Ball 1993). The purpose of this review is to summarize and update our knowledge on the ultrastructure and biology of piscine coccidia and to examine, in light of this new knowledge, our concept of the piscine coccidia's phylogenetic origin and affiliations.

HOST-PARASITE RELATIONSHIPS

Extraintestinal infection

Extraintestinal infections are not exceptional among piscine hosts, as they are among avian and mammalian ones (Overstreet 1981). Ultrastructural data are now available from several extraintestinal species: *Calyptospora funduli* (Hawkins et al. 1983a, b, 1984), *Goussia clupearum* (Morrison & Hawkins 1984), infecting hepatocytes; *Eimeria sardinae* infecting testes (Morrison & Hawkins 1984); *G. spraguei* (Morrison & Poynton 1989) and an undescribed species (Lukes 1993) infecting the kidneys; *G. cichlidarum* infecting the swimbladder epithelium (Paperna et al. 1986, Kim & Paperna 1993a) and *G. gadi* infecting the swimbladder's loose connective tissue (Morrison et al. 1993a, b).

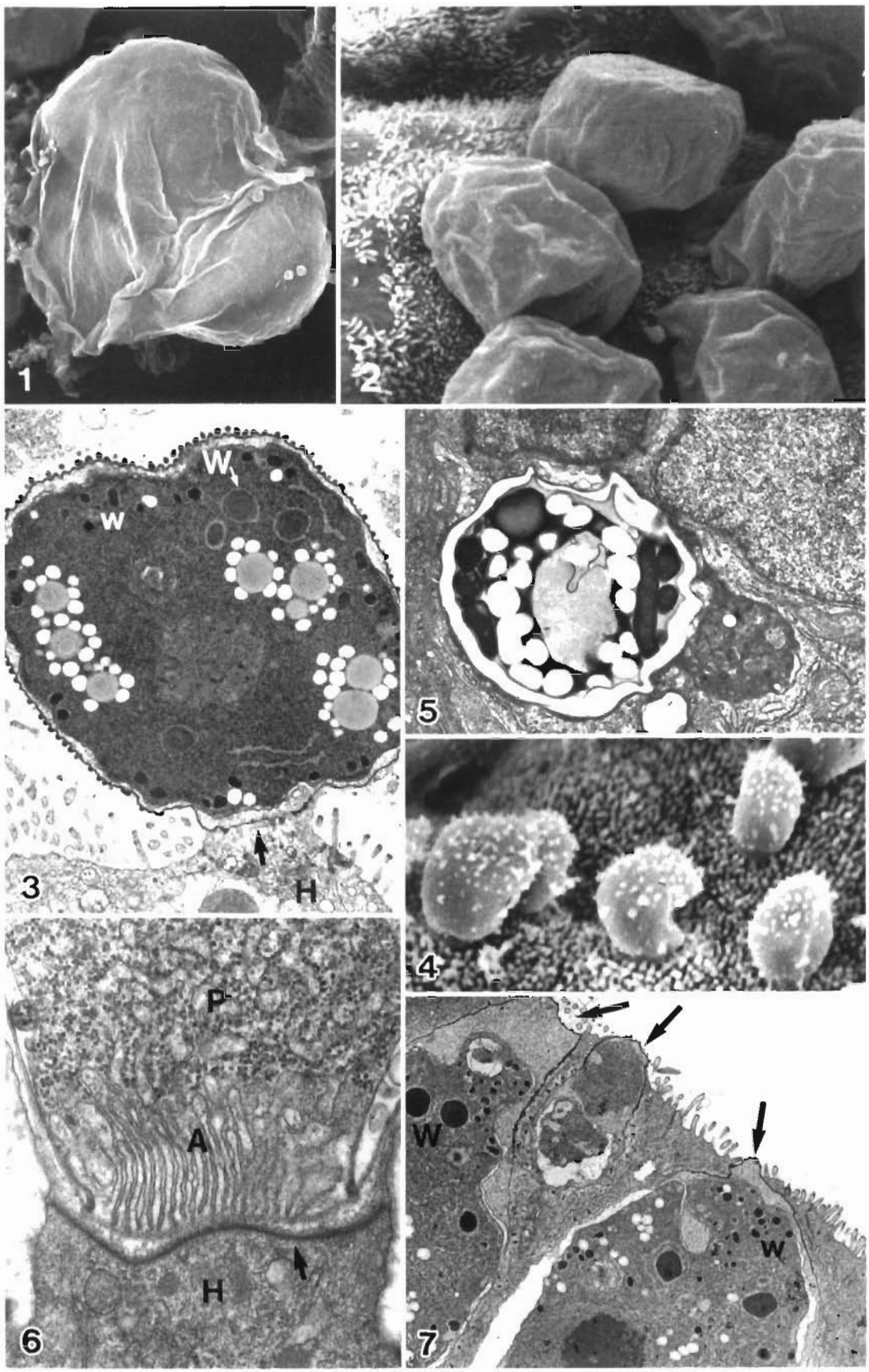
Except for members of the genus *Calyptospora* which are all intrahepatic and, thus far, all species from American fishes (Overstreet et al. 1984, Cheung et al. 1986, Bekesi & Molnár 1991, Azevedo et al. 1993), there is no common structural pattern separating the extraintestinal species as a group from species found in the gut epithelial cells. Endogenous sporogony is common to all extraintestinal species, but it could be the inevitable consequence of their location. It is also common among intestinal species (Dyková & Lom 1981). In *Goussia cichlidarum* and *G. gadi* sporozoites or trophozoites, free or within macrophages, reach the swimbladder via the bloodstream, and in *G. cichlidarum*, via the rete mirabilis into the lining epithelium (Kim & Paperna 1993a, Morrison et al. 1993a). In *G. subepithelialis*, sexual stages spread, during the normal course of infection, from the gut epithelium into the lamina propria (Steinhagen et al. 1990). In *Eimeria vanasi*, when intestinal infection declines, asexual stages may be found in the macrophages of the lamina propria, where merozoites are further divided by endodyogeny (author's pers. obs). During heavy infections of *E. vanasi*, merogonal stages were found in the intestinal serosa, the pancreas and the liver, in the latter organ macrogamonts become established too (Kim 1992).

Epicytoplasmic infection

Epicytoplasmic infections are established either through penetration beneath and then lifting of the brush border of the host cell (Paperna 1991, Kim & Paperna 1992), or, alternatively, while becoming enclosed on the host cell surface by microvilli-derived extensions (Lukes 1992). The parasitophorous vacuole (PV) containing the growing parasites is covered by the host cell apical wall as it expands above the epithelium (Fig. 2). The PV wall merges with the apical host cell wall (which usually loses its microvilli in the process) to form a parasitophorous envelope (PE) (Fig. 3). The most notorious epicytoplasmic infection is that of the genus *Cryptosporidium*. *Cryptosporidium* sp. of Landsberg & Paperna (1986) infecting cichlid fish differs from those found in higher vertebrates in that the villi are retained on the PE (Fig. 4) and, more important, at the end of its developmental cycle the oocyst enters the gut epithelial cell to sporulate (Fig. 5; Landsberg & Paperna 1986, Paperna 1987). *Cryptosporidium* sp. establishes a unique elaborate juncture (in the form of an attachment organ) with the host cell cytoplasm (Fig. 6). This, as well as its peculiar nonflagellated microgamont, justifies its exclusion from the eimeriid coccidia. *Cryptosporidium* sp. is therefore not related, as was previously suggested (Levine 1984), to eimeriid epicytoplasmic coccidia. Among vertebrate hosts, epicytoplasmic eimeriid coccidia have only been found to date among reptiles (the genus *Acroeimeria*; Paperna 1989, Paperna & Landsberg 1989) and fish (Dyková & Lom 1981). Noteworthy is also the epicytoplasmic position of terrapine haemogregarines in the epithelial cell of the leech gut (Siddall & Desser 1990). There is no phylogenetic relationship between reptilian and piscine epicytoplasmic species, each has features in common with those of the intracytoplasmic coccidia of their respective host group (Paperna 1989). There is, however, great ultrastructural variability among the piscine species studied to date (see Figs. 3, 9, 13 & 23). Dyková & Lom (1981) proposed a new genus, *Epieimeria*, to contain the epicytoplasmic piscine coccidia. Of all the epicytoplasmic species, however, only *E. anguillae* from eels (Molnár & Baska 1986) and *E. isabellae* from conger eels (Daoudi et al. 1985) seem to be congeneric. Sporocysts of *Epieimeria* spp. have an apical aperture with a plug reminiscent of a Stieda body (Lom & Dyková 1982). The remaining species are *Eimeria* s. l. *vanasi* and species of *Goussia*, with bivalved sporocysts [ultrastructurally studied: *G. zarnowskii* (Jastrzebski & Komorowski 1990), *G. cichlidarum*, *G. spraguei* (Morrison & Poynton 1989), *G. pannonica* (Lukes 1992), *G. trichogasteri* (Kim & Paperna 1993b) and an unnamed species (Lukes 1993)]. Despite having similar bivalved sporocysts, *G. cichlidarum* and *G. spraguei* seem to differ ultrastructurally, as well as in the nature of their parasitophorous junction, from the rest.

Eimeria vanasi forms both epicytoplasmic and intracytoplasmic endogenous generations, which include both merogonous and gamogonous stages. Moreover, ultrastructural studies reveal an active transition from intracellular to epicytoplasmic positions in the host cell (Fig. 7) (Paperna 1990, 1991, Kim & Paperna 1992).

Figs. 1 to 7. Fig. 1. Sporulated oocyst of *Goussia cichlidarum*. Note transparent oocyst wall. SEM, ×4500. Fig. 2. Gamonts of *G. cichlidarum* in the swimbladder of *Oreochromis aureus*. SEM, ×8000. Fig. 3. Epicytoplasmic macrogamont of *Eimeria vanasi* enclosed in a parasitophorous envelope, from intestine of *O. aurea* × *niloticus*. H: host cell; w, W: type 1 (w) and 2 (W) wall-forming bodies; arrow: juncture zone. TEM, ×9200. Fig. 4. *Cryptosporidium* sp. of Landsberg & Paperna (1986), in stomach of *O. aureus* × *niloticus*. SEM, ×17 000. Fig. 5. *Cryptosporidium* sp. in Landsberg & Paperna (1986), oocyst in stomach mucosa. TEM, ×25 000. Fig. 6. Juncture zone (arrow) and attachment organ (A) of *Cryptosporidium* sp. in Landsberg & Paperna (1986) (P); H: host cell. TEM, ×72 000. Fig. 7. *E. vanasi* macrogamonts in transition from intracytoplasmic to epicytoplasmic position. Arrows: emerging tips of the parasitophorous vacuole (PV); w, W: type 1 (w) and 2 (W) wall-forming bodies. TEM, ×5100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Intranuclear infections

Several species of piscine coccidia (with both *Eimeria* s. l. and *Goussia*-type sporocysts) undergo their entire endogenous development in the nucleus of the host cell (Daoudi et al. 1987, Davis & Ball 1993). In *E. vanasi*, intranuclear infections (Fig. 8) (with merogonal stages and gamonts) occur concurrently with intracytoplasmic and epicytoplasmic stages. The conspecificity of these forms has not been definitely concluded (Paperna 1991).

Endogenous sporulation

Most coccidia leave fish in a sporulated stage. Under certain circumstances, such as enhanced evacuation, oocysts of the same species may be defecated unsporulated. At the same time intestinal conditions do not inhibit sporulation of species normally defecated unsporulated, where evacuation and release of oocysts is delayed (Molnár 1984, Paperna unpubl. obs).

Sporulation in a number of coccidia (e.g. *Goussia carpelli*) starts while the oocyst is still intracellular in the intestinal epithelium (Lom et al. 1991) and often only sporocysts emerge from such *in situ* sporulated oocysts.

STRUCTURE AND DIFFERENTIATION

The parasitophorous vacuole

Special structures, or organelles, found in the wall of the PV seem to be involved in metabolic exchange between the PV and the host cell cytoplasm. The entire contact zone between *Goussia cichlidarum* and the epithelial host cell lining the swimbladder is a hemidesmosome (Figs. 9 & 10; Paperna et al. 1986). The elaborate tubular system which drains into numerous funnels along the wall of the PV in intracytoplasmic merogonal stages and gamonts of *Eimeria vanasi* (Fig. 11; Paperna & Landsberg 1987) has not yet been found in other piscine coccidia. Also unique to *E. vanasi* is a multilayered PV (Fig 12) which displaces the tubular system funnels during certain developmental phases (Paperna & Landsberg 1987, Paperna 1991). Invaginations of various sizes and numbers, usually containing an electron-dense core or deposit, occur at the adjoining host cell cytoplasm/PV zone of epicytoplasmic species (*Epieimeria* spp., *G. zarnowskii*) or epicytoplasmic stages (in *E. vanasi*) (Fig. 3). In the latter species, the PV membrane of juvenile parasites sends off numerous invaginations and fibrillar bundles into the host cell cytoplasm, thus resembling an inverted brush border (Figs. 13 & 14). In spite of the similarities between funnels and the various types of invaginations, they need not necessarily be homologous. An elaborate system of folds occurs in the PE of epicytoplasmic stages of *E. vanasi* (Fig. 3) and of some undescribed marine species (Boulard & Blanc 1985).

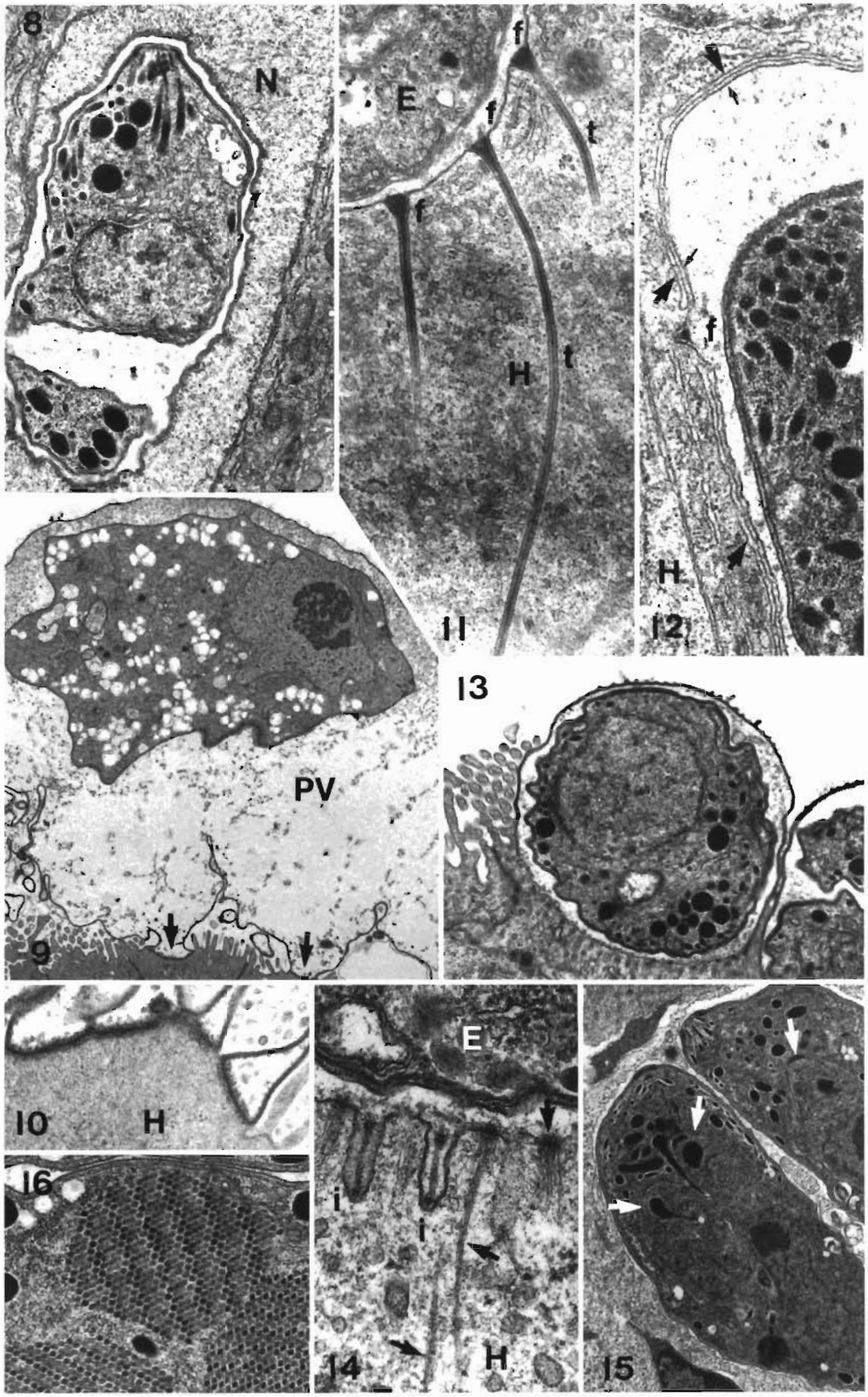
Endodyogeny

Endodyogeny, characteristic of asexual reproduction in cyst-forming coccidia [*Toxoplasma*, *Sarcocystis* and *Cystoisospora* (syn. *Levinea*); Dubey (1977), Ferguson et al. (1980)], appears to be the common form of asexual division in successive generations of piscine coccidian merozoites (Fig. 15) prior to their differentiation into meronts (Paperna 1991, Kim & Paperna 1992, Morrison et al. 1993a). Endodyogeny also takes place in trophozoites of *Goussia cichlidarum* before, or as soon as, they enter the swimbladder (Kim & Paperna 1993a). In *Eimeria vanasi* also merozoites enclosed within macrophages in the lamina propria undergo endodyogeny.

Merogony

Among all the ultrastructurally studied piscine coccidia to date, endomerogony, or rather endopolygeny, is the only demonstrated form of merogony in *Epieimeria anguillae*, *Goussia aculeati*, *G. carpelli* (Steinhagen 1991a) and *G. cichlidarum* (Kim & Paperna 1993a). In *G. iroquoina*, ectomerogony occurs prior to subsequent generations of endomerogony (Paterson & Desser 1981a).

Figs. 8 to 16. Fig. 8. Intranuclear *Eimeria vanasi* trophozoite. N: host-cell nucleus. TEM, ×14 800. Fig. 9. Young *Goussia cichlidarum* macrogamont; arrows point at PV-host cell junctures. TEM, ×4900. Fig. 10. Enlarged view of the PV-host cell juncture. H: host cell. TEM, ×20 000. Fig. 11. Tubuli (t) and funnels (f) connected to *E. vanasi* (E) PV wall; H: host cell. TEM, ×22 800. Fig. 12. *E. vanasi* PV wall (fine arrows) with residual funnel (f) doubled through addition from the endoplasmic reticulum (bold arrow); H: host cell. TEM, ×25 000. Fig. 13. Epicytoplasmic *E. vanasi* trophozoites with its specialized invaginated PV juncture. TEM, ×10 500. Fig. 14. Enlarged view of *E. vanasi* (E) PV juncture with invaginations (i) and fibrillar bundles (arrows); H: host cell. TEM, ×46 200. Fig. 15. Two *E. vanasi* merozoites undergoing endodyogeny (arrows). TEM, ×7500. Fig. 16. Crystalline organelles in *E. vanasi* merozoite. TEM, ×24000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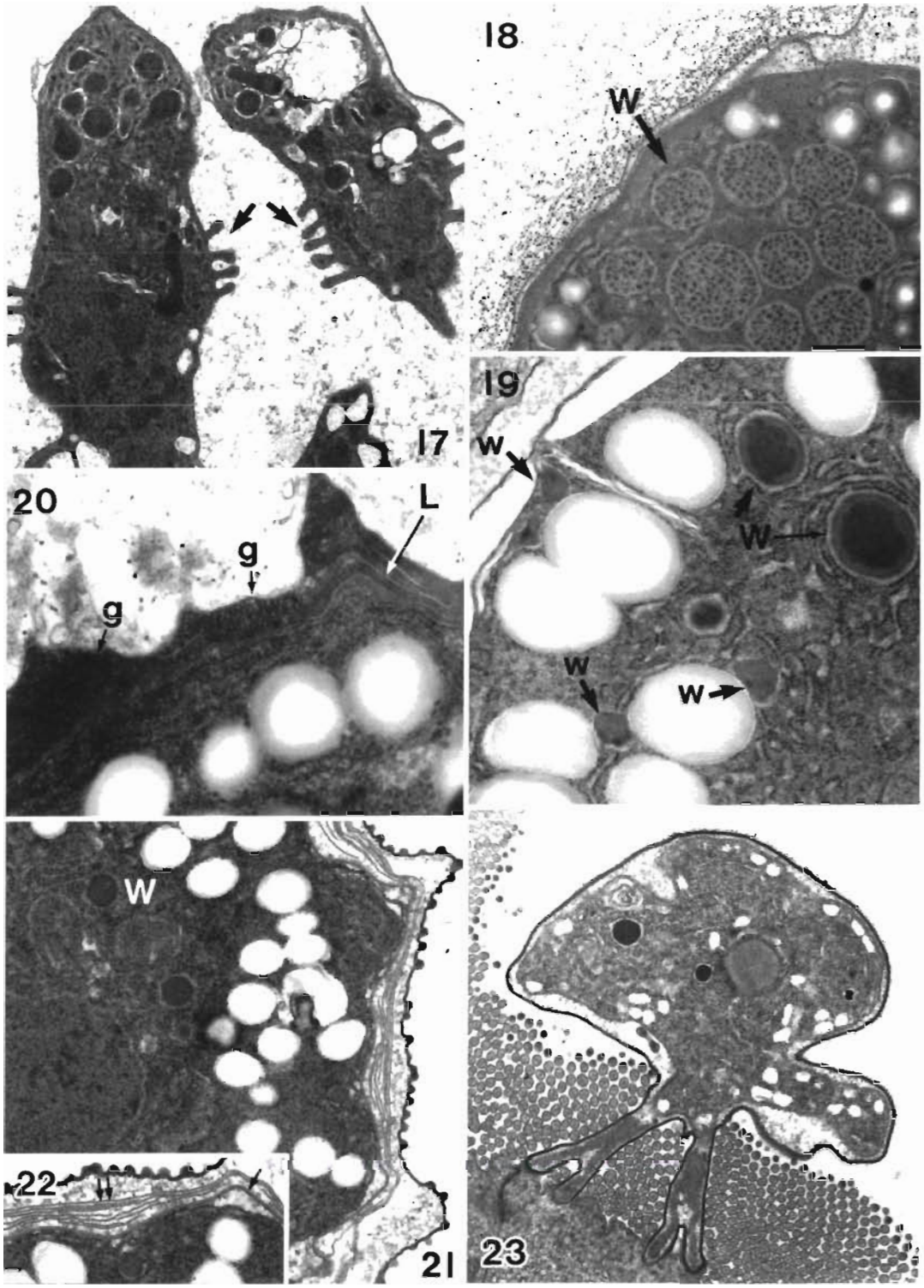

Figs. 17 to 23. Fig. 17. *Goussia cichlidarum* with pellicular projections (arrows). TEM, ×15 000. Fig. 18. Granular WF2-like organelles (W) in *G. cichlidarum* macrogamont. TEM, ×11 250. Fig. 19. Small WF1-like (w) and large WF2-like (W) organelles in intracytoplasmic macrogamont of *Eimeria vanasi*. TEM, ×22 500. Fig. 20. Forming oocyst wall in *G. cichlidarum* sporoblast. g: granular aggregates; L: laminae. TEM, ×27 000. Fig. 21. Forming wall in *E. vanasi* epicytoplasmic oocyst. W: WF2-like organelles. TEM, ×15 800. Fig. 22. Enlarged view of oocyst wall from Fig. 21, showing wall membrane (arrow) and overlaid lamellae layer (2 arrows). TEM, ×20 000. Fig. 23. *Goussia trichopteri* (young macrogamont) from intestine of a gourami *Trichogaster pectoralis*. TEM, ×9500

Refractile and crystalline organelles

Sporozoites, and some merozoite stages of piscine coccidia, possess either a refractile body, characteristic of all eimeriid coccidia (Hammond et al. 1970, Chobotar & Scholtyseck 1982), or a crystalline body, characteristic of *Cystoisospora*, *Sarcocystis*, some invertebrate coccidia and all haemosporidia (Trefiak & Desser 1973, Chobotar & Scholtyseck 1982). Refractile bodies were found in *Eimeria sardinae* and *Goussia clupearum* (Morrison & Hawkins 1984), *G. carpelli* and *G. subepithelialis* (Lom et al. 1991, Steinhagen 1991b), and *Calyptospora funduli* (Hawkins et al. 1983a). Crystalline bodies were found in *G. cichlidarum* (Paperna & Landsberg 1985), *G. spraguei* (Morrison & Poynton 1989; also with refractile bodies?), merozoites of *G. gadi* (Morrison et al. 1993a) and of *E. vanasi* (Fig. 16; Kim & Paperna 1992).

Pellicular projections in merozoites

Pellicular projections occur in merozoites of *Goussia cichlidarum* (Kim 1992; Fig. 17). They are unknown in other coccidians, but occur in intraerythrocytic stages of haemogregarines (Desser & Weller 1973, I. Paperna & Y. Boulard unpubl.) in amphibians and reptiles.

Wall-forming bodies

Macrogamonts of all terrestrial vertebrate-host eimeriid coccidia with hard-walled oocysts contain wall-forming organelles (WF1 and WF2), which provide the material for the outer and inner oocyst walls (Scholtyseck et al. 1971, Chobotar & Scholtyseck 1982). One might expect piscine coccidia, which do not form hard oocyst walls, to be without wall-forming bodies. Indeed, *Epieimeria anguillae* and *E. isabellae* lack any organelles resembling wall-forming bodies. The identification of wall-forming bodies in *Calyptospora funduli* (Hawkins et al. 1983b) should be reexamined. In *Goussia iroquoina* (Paterson & Desser 1981b), *Eimeria laureleus*, *G. aculeati*, *G. carpelli* and *G. pannonica* macrogamonts contain electron-dense bodies which are involved in neither oocyst nor sporocyst wall-formation (Desser & Li 1984, Paterson & Desser 1984, Jastrzebski 1989, Lom et al. 1991, Lukes 1992). The same applies to the large globular osmiophilic granules seen in *G. spraguei* (Morrison & Poynton 1989) and the peripherically arranged dense inclusions of *G. subepithelialis* (Steinhagen et al. 1990). It has been suggested that these organelles, which persist throughout sporulation, become sporozoite refractile bodies (Paterson & Desser 1984). Similar dense bodies found in *G. zarnowskii*, however, have been proposed as the source of material deposited between the membranes of the forming oocyst wall. Morrison & Hawkins (1984) report the presence of WF2-like organelles, located within endoplasmic reticulum (ER) cisternae, in both *G. clupearum* and *Eimeria sardinae*. Similar granules within ER cisternae are found in *G. trichopteri* (Kim & Paperna 1993b; see Fig. 23). WF2-like organelles located in ER cisternae, occurring in the macrogamonts of *G. cichlidarum* (Paperna et al. 1986), seem to contribute their granular contents to the consolidating oocyst wall (Fig. 18) (Paperna & Landsberg 1985). So far, among piscine coccidia, only *E. vanasi* macrogamonts contain organelles which are structurally reminiscent of the WF1 and WF2 in higher vertebrate coccidia (Figs. 3, 7 & 19). WF1-like organelles seem to contribute material during the early stages of wall formation, while WF2-like organelles persist until later stages of wall formation, with their ultimate fate remaining unknown (Kim & Paperna 1992).

Wall formation

In *Goussia iroquoina*, *Eimeria laureleus*, *G. carpelli* and *G. spraguei*, 1 or more membranes, formed after macrogamete fertilization, together with the closely applied PV, make up the oocyst wall: a thin membranous or laminated envelope which degenerates when the sporocysts mature (Desser & Li 1984, Paterson & Desser 1984, Lom et al. 1991). The dense bodies do not seem to participate in the process and may be found beneath the wall of the sporulating oocyst (Davies 1990). Detached oocysts often remain embedded in the residue of their host cell. In the intrahepatocytic *Calyptospora funduli* the oocyst wall is particularly thin, 2-layered, with a finely deposited inner layer (Hawkins et al. 1984). In *G. zarnowskii* wall formation, 3 additional distinct membranes are formed above the triple-layered zygote wall and a finely granulated material is deposited between the second and third membranes, possibly from the wall-forming-like bodies (Jastrzebski & Komorowski 1990). In *G. cichlidarum* the outer cytoplasmic layer of the zygote becomes the oocyst wall. Granular material, apparently of WF2 origin, aggregates in the peripheral cytoplasm which subsequently consolidates into a laminated layer (Fig. 20). However this layer eventually disintegrates, becoming the fragile membrane, seen in the other piscine coccidia, when sporocysts are formed (Paperna & Cross 1985, Paperna & Landsberg 1985). In *E. vanasi*, an outer multilamellated layer is formed, which, together with a deposited substance, consolidates into a wall with an outer and inner layer, reminiscent of higher vertebrate coccidia oocyst walls (Figs. 21 & 22) (Kim & Paperna 1992).

TRANSMISSION

There seem to be several options for the transmission of piscine coccidia: via oral contamination (predation or necrophagia) and via intermediate hosts. The intestinal species *Goussia iroquoina*, *G. subepithelialis*, *G. carpelli* and *Eimeria vanasi* and the swimbladder species *G. cichlidarum* were transmitted by oral inoculation of sporulated oocysts or by allowing fish to feed on contaminated feces and swimbladders containing sporulated oocysts (Marincek 1973, Paterson & Desser 1981a, Steinhagen & Körting 1988, Kim 1992, Kim & Paperna 1993a). Necrophagia seems to be an important route of transmission not only for extraintestinal but also for intestinal coccidia which sporulate endogenously (Molnár 1984). Steinhagen & Körting (1988) were able to transmit *G. subepithelialis* and *G. carpelli* via paratenic hosts, namely tubificid oligochaetes. Sporozoites excysted from ingested oocysts, invaded and survived in the gut cells (Steinhagen 1991b). Fournie & Overstreet (1983) demostrated that transmission of *Calyptospora funduli* requires an obligatory intermediate host, the grass shrimp *Palaemonetes pupio*. Heteroxenous transmission has been demonstrated in moray eel coccidia via mysidacid shrimp (Landau et al. 1975).

CONCLUDING REMARKS

The wide variability among piscine coccidia in structure, host-parasite relationships and biology indicates a polyphyletic origin. The best example of this is the finding of both crystalline and refractile organelles in the sporozoites and merozoites of piscine coccidia. Alternatively, or complementarily, this diversity may be an expression of a lower degree of evolutionary specialization, as compared to the high structural and developmental conformity among the very specialized avian and mammalian monoxenous eimeriid coccidia. The heteroxenous development demonstrated in some coccidia may serve as further proof of a lower level of specialization, following Landau's (1982) postulation that monoxenous coccidia originated from ancestral heteroxenous forms. Many of the structural features found among piscine coccidia also occur in the heteroxenous cyst-forming coccidia *Sarcocystis* and *Cystoisospora*: fragile oocyst; valved sporocysts lacking Stieda bodies which rupture along sutural lines; crystalline bodies in sporozoites and merozoites instead of refractile ones; a PV wall which is either multilayered, or has funnels or invaginations, endodyogeny as a stage in merogonous development and endopolygeny as the main form of merogony (Pelster 1973, Dubey 1977, Ferguson et al. 1980). The occurrence of merozoites, dividing by endodyogeny inside migratory macrophages in extraepithelial sites during chronic stage of infection with *Eimeria vanasi*, recalls a chronic form of *Toxoplasma* infection (Dubey 1977), and 'cystozoites' of haemogregarines (Kirmse 1979). Endodyogeny has only rarely been observed among higher vertebrate monoxenous eimeriid coccidia (Danforth & Hammond 1972). In addition to having crystalline rather than refractile bodies, other shared features with haemogregarines are the pellicular projections found also on intraerythrocytic stages of haemogregarines (Desser & Weller 1973), and the epicytoplasmic position which also characterizes the association between gamonts and the gut cells of the leech (Siddall & Desser 1990). The existence of similar characteristics, however, does not necessarily imply a direct phylogenetic relationship between the cyst-forming and piscine coccidia. An alternative possibility could be to regard all of the above-listed traits as ancestral characteristics of coccidia which, according to Landau's (1982) postulation, were lost or replaced by new devices (such as the hard 2-layered oocyst wall or the sporocyst Stieda and Substieda bodies) in the process of evolutionary specialization. The epicytoplasmic form of infection could also be regarded as one of the coccidian features eliminated during the evolution of mammalian and avian eimeriid coccidia. The crystalline body which occurs in invertebrate coccidia as well as in haemosporidia seems to be older than the refractile body characteristic of eimeriid coccidia. Other apparently ancient organelles are the pellicular projections found in some merozoites, and otherwise found only in haemogregarines and the invagination of the PV, which in addition to *Cystoisospora* spp. were reported in exoerythrocytic stages of tortoise *Haemoproteus metchnikovi* (Sterling & de Giusti 1972).

The absence of true wall-forming bodies sets apart all piscine coccidians from those of reptilian, avian and mammalian hosts, including the cyst-forming ones (which display at least one of the two; Dubey 1977, Dubey et al. 1989). A recent study of frog coccidia (Paperna & Lainson 1995) demonstrates their similarities with piscine coccidia, e.g. the occurrence of dense organelles rather than wall-forming types, and oocysts with soft, disaggregating walls which complete sporulation in the host tissue prior to evacuation.

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