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# Morphology and biology of *Goussia carpelli* (Protozoa: Apicomplexa) from the intestine of experimentally infected common carp *Cyprinus carpio*

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ABSTRACT: Development of *Goussia carpelli*, a coccidian parasite of intestinal epithelial cells of carp, was studied in experimentally infected hosts. Merogony, gamogony and oocyst formation were described by light microscopy. The course of development was highly influenced by temperature. At 20°C development was completed in 9 to 10 d post exposure (PE). At low temperature morphological development and oocyst formation were markedly retarded. At 15°C infected carp released sporulated oocysts 18 to 19 d PE, at 12°C 32 to 35 d PE and at 8 to 10°C 37 d PE. Carp aged 4 wk to 4 yr were susceptible to *G. carpelli* infections.

## INTRODUCTION

*Goussia carpelli* (Léger & Stankovitch, 1921) Dyková & Lom, 1983, formerly known as *Eimeria carpelli*, is a well known parasite of intestinal epithelial cells of carp and goldfish from Europe and North America and widespread among hatchery populations of these fishes. Information on the biology and morphological features of developmental stages of *G. carpelli* has been derived from studies on naturally infected fishes. Seasonal dynamics of infection were reported by Zaika & Kheisin (1959), Ivasik & Kulakovskaya (1959), Marinçek (1965) and Kocylowski et al. (1976) from European carp hatcheries, and more recently from cyprinids of a river basin by Alvarez-Pellitero & Gonzalez-Lanza (1986). Morphological features of developmental stages were depicted and described briefly in the original description of the parasite (Léger & Stankovitch 1921) from carp and by Kent & Hedrick (1985) for specimens from goldfish. Ultrastructural observations on oocysts and sporocysts (Lom & Dyková 1982) showed that *G. carpelli* sporozoites are enclosed in a 2-valved sporocyst shell with a prominent suture line. Therefore *G. carpelli* was placed into the re-established genus *Goussia* Labbé (Dyková & Lom 1983).

In the present study, morphological development and biology of *Goussia carpelli* from experimentally infected carp are described for the first time. Merogony, gamogony and oocyst formation of *G. carpelli* in intestinal epithelial cells from carp are described by light microscopy, and the impact of temperature and host age on the course of development is examined.

## MATERIALS AND METHODS

Common carp *Cyprinus carpio* L. in this study were reared in our laboratory from fertilized eggs of an artificially bred stock obtained from the Department of Experimental Animal Morphology and Cell Biology, Agricultural University, Wageningen, The Netherlands. Eggs and carp fry were kept in glass tanks containing aerated well water at 21 to 23°C, and fed on *Artemia salina* larvae once or twice a day. Carp aged 7 and 11 mo and 4 yr were kept in glass tanks with aerated tap water and fed on pelleted dry food (Rheinkrone Kraftfutterwerke, Wesel, FRG). Before the carp were used for infection experiments the intestines of 5 to 8 carp were examined for the presence of *Goussia carpelli* oocysts.

The carp were infected experimentally with *Goussia carpelli* by fecal contamination as reported previously

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(Steinhagen & Körting 1988). To study the development of *G. carpelli,* 15 to 20 mm *Cyprinus carpio* were infected experimentally and kept at 20 °C. Beginning with Day 1 post exposure (PE), 3 to 4 carp were dissected every day, the digestive tract was removed and opened lengthwise. From the anterior part of the intestine a 2 × 5 mm region was removed, smeared on a glass slide, air-dried, fixed with methanol, and stained with Giemsa's stain. The remaining part of the intestine was fixed in 4 % (w/v) formaldehyde in phosphate buffer pH 7.2, dehydrated in graded ethanol and embedded in glycol methacrylate resin (Technovit, Kulzer, Wehrheim, FRG). Sections 2 to 3 µm thick were stained with hematoxylin and eosin or with Giemsa's stain (Gerrits 1985). Photomicrographs were taken with a Zeiss Photomicroscope III using Ilford Pan F film. Measurements were taken with an ocular micrometer from fresh oocysts and developmental stages from smear preparations and histological sections.

To study the influence of temperature on the development of *Goussia carpelli,* carp were placed into a contaminated tank for 12 h at 20 °C, then transferred to clean tanks with aerated tap water and sterile washed sand as bottom substrate (Paterson & Desser 1982), and kept at 8 to 10, 12, 15, 20 and 30 °C. From the groups at 15, 20 and 30 °C, 3 to 4 carp were dissected and processed daily, from those at 8 to 10 and 12 °C every other day.

The infectivity of *Goussia carpelli* oocysts produced at 12, 15, 20 and 30 °C was examined using tanks from the above-mentioned temperature experiments. Infected carp were removed from these tanks after sporulated oocysts had been released. Uninfected carp were then transferred to these contaminated tanks, still maintained at the previous temperatures.

The susceptibility of carp from different age groups to experimental infection with *Goussia carpelli* was examined by exposing carp aged 4 wk, 7 and 11 mo, and 4 yr to *G. carpelli* oocysts.

## RESULTS

Common carp, exposed to oocysts of *Goussia carpelli* at 20 °C, harboured merogonic stages in intestinal epithelial cells up to Day 8 PE; gamogony was observed during Days 7 to 9 PE, and sporogony was completed at Day 9 to 10 PE. Merogony, gamogony and oocyst formation occurred in the anterior third of the intestine. The first merogonic stages, seen in experimentally infected carp, resembled small globular cells (3 to 5 µm in diameter) with an eccentric nucleus, measuring 1.6 to 3.0 µm ($n$ = 25). Stages with 2 to 8 nuclei were seen in Giemsa-stained preparations. Differentiated meronts, in histological cross-sections, had a diameter of 5 to 8 µm and contained 8 to 14 merozoites ($n$ = 25) (Fig. 1 inset). In Giemsa preparations these merozoites measured 6 to 9 by 1 to 2 µm with a nucleus of 1.6 to 3.1 by 1.0 to 2.0 µm (Fig. 2). On histological sections merogonic stages were seen in the apical region of epithelial intestinal cells (Fig. 1). Light microscopic observations of merogonic stages from consecutive days did not reveal morphological differences which could allow separation into different meront generations.

Early gamonts appeared in the intestinal epithelial cells mainly in the nuclear region (Fig. 3), and could not be distinguished from early merogonic stages. Young microgamonts, found mainly in the apical cell region, contained many peripherally arranged nuclei, which later gave rise to microgametes (Figs. 4 and 5). During differentiation, the microgamonts developed from elongated stages to nearly global forms, measuring 5 by 5 to 18.7 by 12 µm ($n$ = 25, measurements taken from histological sections). During maturation of macrogamonts the cell shape also changed. Young macrogamonts were irregular in shape and had basophilic cytoplasm (Fig. 6). Maturing macrogamonts contained masses of non-staining inclusions and some densely stained global bodies. In histological sections maturing macrogamonts had an oval to rounded shape, measuring 6 by 12 to 12.5 by 24 µm ($n$ = 25). Developing oocysts were mainly found in the subnuclear region of the intestinal epithelial cells and lamina propria of the intestinal tissue (Fig. 8). Oocyst sporulation gave rise to 4 sporocysts each with 2 sporozoites (Figs. 9 and 10) and occurred within the host cell, which degenerated (Fig. 9). Sporulating oocysts often lay close together with the degenerating host cells forming large clusters (Fig. 9), on wet mount preparations visible as 'yellow bodies'. Measurements of fresh oocysts are presented in Table 1.

Temperature had a clear impact on the development of *Goussia carpelli.* As shown in Table 2, oocyst formation was markedly retarded when experimentally infected carp were kept at temperatures of 15 °C or less. Merogony, gamogony and oocyst formation were influ-

Figs. 1 to 9. *Goussia carpelli.* Fig. 1. Histological section of carp intestine infected with *G. carpelli* showing meronts apical to the host cell nuclei. M: meront; N: host cell nucleus. Inset: Meront, cross section. Fig. 2. Merozoites, impression film. Fig. 3. Intestine with numerous early gamonts, basal to the host cell nuclei. G: gamont. Fig. 4. Early microgamont with peripherally arranged nuclei (arrows). Fig. 5. Advanced microgamonts with microgametes (MG). Fig. 6. Young macrogamont of irregular shape (arrows). Fig. 7. Mature macrogamete. Fig. 8. Oocyst formation in lamina propria cells of infected carp. O: sporulating oocysts. Fig. 9. Cluster of sporulated oocysts, phase interference optics, fresh preparation. All figs. plastic section, bar = 10 µm, except Fig. 2: impression film, Giemsa's stain, bar = 5 µm

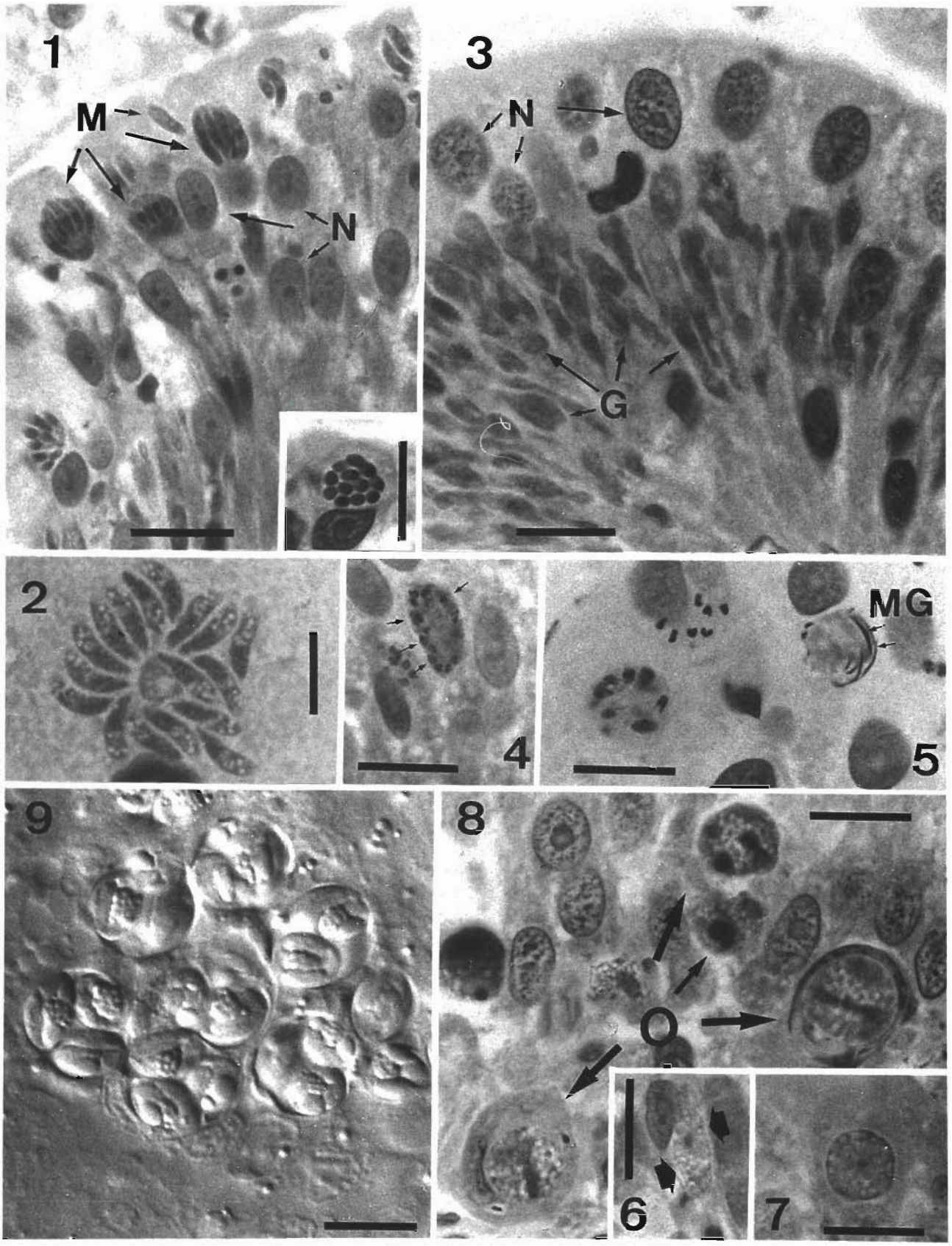
1
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Table 1. *Goussia carpelli*. Measurements of sporulating stages of specimens from the intestine of *Cyprinus carpio*

| Sporulating stage | Léger & Stankovitch (1921) | Measurements from this study[a] |
|---|---|---|
| Oocyst (diameter) | 13–14 | 10.4–13.5<br>11.6±0.8 |
| Sporocyst | 8×6 | 7.3–10.4×5.2–6.2<br>8.1±0.8×5.5±3.4 |
| Sporozoite | 6–7 | 7.3–9.4×1.5–2.1<br>8.5±0.5×1.8±0.3 |
| Residual body | ng | 2.1–3.1<br>2.7±0.4 |
| Immature oocyst | ng | 8.3–11.4<br>9.6±0.8 |
| Sporoblasts (diameter) | ng | 5.2–7.3×4.2–5.2<br>6.3±0.7×4.6±0.5 |

[a] Measurements in µm; first line: range, second line: mean ± standard deviation. Measurements taken from fresh material, $n = 25$
ng: not given

Fig. 10. *Goussia carpelli* oocyst showing sporocysts, sporozoites and residual bodies. Bar = 5 µm

Table 2. *Goussia carpelli*. Influence of temperature on the appearence of developmental stages in experimentally infected *Cyprinus carpio*. Given in days post exposure when the developmental stages were observed for the first time. Carp: 15 to 25 mm body size, 4 wk old. Range of measurements from 3 different experiments

| Developmental stages | 8–10 °C | 12 °C | 15 °C | 20 °C | 30 °C |
|---|---|---|---|---|---|
| Schizonts | nd | 4 | 4 | 3 | 2 |
| Meronts | nd | 4 | 5 | 3 | 2 |
| Gamonts | nd | 23–25 | 15 | 7–8 | 5 |
| Zygotes | nd | 25 | 17 | 8–9 | 6 |
| Oocysts, sporoblasts | nd | 29 | 17 | 9–10 | 6 |
| Sporulated oocysts | 37 | 32–35 | 18 | 9–10 | 7 |

nd: not done

enced by temperature in a similar way (Table 2). However, in all experiments performed at temperatures from 8 to 30 °C, oocyst formation was completed. Oocysts produced at 12, 15, 20 and 30 °C were able to infect laboratory-reared carp.

*Goussia carpelli* infected laboratory-reared carp of ages 4 wk, 7 and 11 mo, and 4 yr.

## DISCUSSION

Research on the biology of piscine coccidia was promoted since Dyková & Lom (1981) and Desser (1981) gave comprehensive overviews of the literature dealing with these parasites. Nevertheless, the development of coccidia from experimentally infected piscine hosts has been described only in a few species: *Goussia cichlidarum* from the swimbladder of cichlid fishes (cf. Landsberg & Paperna 1985), *Goussia iroquoina* from the intestine of the fathead minnow *Pimephales promelas* (cf. Paterson & Desser 1982), *Goussia subepithelialis* from the intestine of carp *Cyprinus carpio* (cf. Marinçek 1973) and *Calyptospora funduli* from the liver of the gulf killifish *Fundulus grandis* (cf. Solangi & Overstreet 1980, Fournie et al. 1985). This situation is mainly caused by the complicated routes of transmission observed in numerous coccidian species from fishes. As Desser (1981) pointed out, it is not possible to transmit several piscine coccidian species to a new host directly, but an invertebrate organism is required as an intermediate host. Investigations on experimental transmission of *Goussia carpelli* to uninfected carp and goldfish *Carassius auratus* were reported by Zmerzlaya (1964), Molnar (1979) and Kent & Hedrick (1985). While Zmerzlaya (1964), described

successful transmission of *G. carpelli* by fecal contamination, Molnar (1979) and Kent & Hedrick (1985) did not observe direct transmission of *G. carpelli* among carp and goldfish, but found some evidence that tubificid oligochaetes might serve as a paratenic host of *G. carpelli*. Experimental transmission of *G. carpelli* to uninfected young carp was recently demonstrated by Steinhagen & Körting (1988). This allowed us for the first time to observe and to describe the development of *G. carpelli* in detail.

Morphological development of *Goussia carpelli* was completed within epithelial intestinal cells and cells of the lamina propria. The initial sites of infection were anterior parts of the intestine. Morphological features of the developmental stages and the site of development, observed in this study, agree with the sketchy information given on *G. carpelli* in the original description (Léger & Stankovitch 1921). Kent & Hedrick (1985), investigating *G. carpelli* infections in goldfish, depicted single meronts and gamonts, but gave no further descriptions of these stages.

At 20 °C merogonic stages of *Goussia carpelli* were observed from Day 3 to Day 8 PE, but in all samples from consecutive days, the measurements of meronts and merozoites, and the number of merozoites developing in a meront, varied within the same ranges. Therefore different merogonic generations, as reported from other piscine coccidia, could not be distinguished by light microscopy. In some species of piscine coccidia second generation meronts produce high numbers of progeny: 125 meronts in *G. cichlidarum* (Landsberg & Paperna 1985) and 50 meronts in *G. subepithelialis* (Marinçek 1973).

In *Goussia carpelli,* internal formation of merozoites, as occurred in the meronts of the final asexual generation of *G. iroquoina* (Paterson & Desser 1982), was not observed. Our data on oocyst formation and morphology are in agreement with the information on *G. carpelli* oocysts given in the original description by Léger & Stankovitch (1921) (Table 1).

Morphological development and oocyst formation of *Goussia carpelli* in experimentally infected carp kept at 20 °C were completed within 9 to 10 d PE. The carp then excreted oocysts, which instantly were able to infect a new host. This enables the parasite to spread rapidly among populations of newly hatched carp in pond environments. Temperatures of about 20 °C are optimal for carp. At this temperature a high feeding activity can be observed (Barthelmes 1981). The short period of development of *G. carpelli* observed at 20 °C shows a close adaption of the parasite to its host. A temperature decrease to 15 °C extended the period of development to 19 d PE, about twice as long as at 20 °C. Lower temperatures led to more delayed development of *G. carpelli* (Table 2). A similar impact of temperature on the development of *G. carpelli* was reported by Zmerzlaya (1966), who observed natural infections of carp with *G. carpelli* in ponds of Russian carp hatcheries. In *Calyptospora funduli* exposed to low temperature, the development of all stages was inhibited, and many forms with abnormalities were observed (Solangi et al. 1982). In all experiments of this study, development of *G. carpelli* was completed and all oocysts formed were infective to carp, even at low temperatures.

Solangi & Overstreet (1980) reported a marked influence of host age on the susceptibility of *Fundulus grandis* for *Calyptospora funduli* infections. While young *F. grandis* were infected at high prevalences and intensities, *C. funduli* only occasionally infected adult fishes. In this study, *G. carpelli* was able to infect carp of all ages tested, from 4 wk to 4 yr. This suggests that older carp can harbour *C. carpelli* infections for some time and may serve as a reservoir of infection for the young of the year.

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