Light Microscopic Study of *Caryospora* sp. (Apicomplexa: Eimeriidae) from Savigny's Agama *Trapelus savignii* (Reptilia: Agamidae) in Egypt

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ABSTRACT

Developmental stages of a suggested new species of *Caryospora* Léger, 1904 were described from the agamid lizards *Trapelus savignii* captured during summer 2019 from the Eastern Desert in Egypt. Overall recorded prevalence is 11.65% (12/103) and sporulation is endogenous. Sporulated oocysts are subspherical (70%) to spherical (30%) measuring 22.29–35.74 × 20.91–32.89 μm (length × width). The oocyst wall is bilayered, smooth, and measures 1.46–2.00 μm. Distinctive features of most oocysts include the presence of a sutured edge-like extension on one side of the oocyst wall and the ability of sporocyst contents to form a condensed curved mass within the sporocyst cavity. One or rarely two polar granules are present. Micropyle and oocyst residuum are absent. Sporocysts are ovoid to ellipsoidal measuring 17.17–22.76 × 12.86–16.76 μm. The morphometric descriptions and measurements of Stieda, sub-Stieda bodies, and sporocyst residuum are determined. Sporozoites are sausage-shaped and measure 5.69–8.59 × 2.08–3.29 μm. Merogonic stages and microgamonts occur mainly in the enterocytes of the small intestine. Endogenous stages are seen mostly with clear large parasitophorous vacuoles. Mature meronts measure 3.95–7.60 × 3.37–7.24 μm and yield 3–11 merozoites with the formation of a residual body. Merozoites are banana-shaped measuring 2.51–3.29 × 0.72–1.20 μm. A microgamont is noticed measuring in 9.53 × 8.42 μm.

INTRODUCTION

Genus *Caryospora* Léger, 1904 is a coccidian parasite infecting mainly snakes and raptors (Upton *et al.*, 1986; Duszynski *et al.*, 2003). *Caryospora* and *Caryospora*-like organisms are characterized by having octozoic monosporocystic oocysts and belong to suborder Eimeriorina, order Eucoccidiorida, class Coccidia, subphylum Conoidasida within the alveolate phylum Apicomplexa (Upton *et al.*, 1986; Votýpka *et al.*, 2017). *Caryospora* represents the third largest genus in species diversity within its family, after *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881, respectively (Duszynski *et al.*, 2003). The occurrence of *Caryospora* spp. in a specific family is a matter of controversy. Traditionally, all *Caryospora* species were placed in family Eimeriidae (Upton *et al.*, 1986). In addition, some authors still classify the reptilian *Caryospora* and avian *Caryospora*-like isolates in the same family (de Santana Miglionico and Viana, 2017; Liu *et al.*, 2020). However, Barta *et al.* (2001) suggested that reptilian *Caryospora* spp. may not belong to Eimeriidae but may closely related to Lankesterellidae due to life history and molecular data similarities.

Oocysts of reptilian *Caryospora* spp. have Stieda and sub-Stieda bodies while avian *Caryospora*-like coccidia lack these structures (Berto *et al.*, 2014). This led some authors to propose a new generic name *Avispora* (Schuster *et al.*, 2016), and later a resurrection of genus *Eumonospora* Allen, 1933 to accommodate the avian *Caryospora*-like organisms within family Sarcocystidae (Chou *et al.*, 2020). Moreover, Megía-Palma (2015) showed through phylogenetic studies that raptorial *Caryospora* is more closely related to cyst-building coccidia while reptilian *Caryospora* is more allied to the intestinal coccidia of genera *Isospora* and *Lankesterella*.

Caryospora species have monoxenous (direct) or facultative heteroxenous (indirect, predator-prey) life cycles with occurring of both merogonic and gamogonic stages in both primary (predator, snakes and raptors) and secondary (prey, rodents) hosts (Upton *et al.*, 1986). Unlike of sporulation exogenously from predators, oocysts sporulate endogenously in prey tissues and sporozoites liberated from these oocysts penetrate other cells forming tissue cysts called caryocysts (Wacha and Christiansen, 1982). Oocyst formed in rodent tissue is membrane-bound or thin-walled containing 8 sporozoites without formation of Stieda, sub-Stieda bodies or true sporocyst wall (Upton and Barnard, 1988). Caryocysts are typically monozoic but sometimes contains up to four sporozoites (Wacha and Christiansen, 1982; Upton and Barnard, 1988). The formation of caryocysts in tissues of rodents suggests the transmission of infection from mouse to mouse (secondary host-secondary host transmission) via cannibalism (Upton *et al.*, 1985).

Genus *Trapelus* Cuvier, 1817 contains terrestrial lizards belonging to family Agamidae within class Reptilia. It is presented by five species in Africa and three species, namely *T. mutabilis*, *T. pallida, and T. savignii*, were recorded in Egypt (Baha El Din, 2006; Wagner *et al.*, 2011). Out of them, the Savigny's Agama *Trapelus savignii* Duméril and Bibron, 1837 is distributed in the northern part of Eastern Desert, Northern Sinai of Egypt and characterized by short, thick heads, equal or nearly equal dorsal scales of the hind limb and a large gular pouch (Baha El Din, 2006). Many intestinal coccidian parasites including *Eimeria* and *Isospora* species have been recorded from reptiles in Egypt (El-Toukhy *et al.*, 2013; Mansour and Abou Shafeey, 2019). However, little is known about the distribution of *Caryospora* species among reptiles of Egypt. The present study aims to investigate and discuss the characteristic features of exogenous and endogenous stages of the present *Caryopsora* species from the Savigny's Agama *Trapelus savignii* in Egypt by light microscopy.

MATERIALS AND METHODS

Experimental Animals:

One hundred and three males and females Savigny's Agama *Trapelus savignii* Duméril and Bibron, 1837 (Reptilia: Sauria: Agamidae) were collected from July to September 2019 from the Eastern Desert in Egypt and transported to invertebrates, Entomology and Parasitology laboratory, Zoology and Entomology Department, Faculty of Science, Helwan University where they were examined for the presence of intestinal coccidia.

Examination of Exogenous Stages (oocysts):

Microscopical oocysts examination of intestinal Coccidia was done by direct fecal smears and flotation technique of the collected feces according to Long *et al.* (1976). The morphology of sporulated oocysts was studied and photographed using a Zeiss research photomicroscope.

Examination of Endogenous Stages:

To study merogonic and gamogonic stages, small pieces of the small intestine, rectum, liver, kidney and skeletal muscles of the infected lizard were taken and fixed immediately in Bouin's solution for at least 12 h at 4 °C and then processed for routine

histological techniques including dehydrating in ascending series of ethanol, clearing in xylene, embedding in paraplast at 62 ºC, sectioning using a rotary microtome. Each change of the procedure takes about 20 minutes. Sections with 3-5 μm thickness were deparaffinized in xylene, hydrated in a descending series of ethanol, stained with H&E stain, covered by a coverslip with Canada balsam and finally examined and photographed using a Zeiss research photomicroscope. All measurements are in micrometres (μm) and presented by length × width (L × W) as the range followed by the mean values ± standard deviation in parentheses.

RESULTS AND DISCUSSION

1- Prevalence:

Twelve out of one hundred and three (11.65%) agamid lizards *Trapelus savignii* were found to be naturally infected with oocysts of the present *Caryospora* species. The rate of natural infection was recorded higher in males (7/58, 12.07%) rather than females (5/45, 11.11%).

2- Description of parasitic stages (Figs. 1–18):

A- Exogenous stages (Figs. 1–9):

I- Description of sporulated oocysts:

Oocysts of the present parasite pass partially or completely sporulated in host feces and hence sporulation is endogenous. The sporulated oocyst contains eight sporozoites that occupying the cavity of a single sporocyst (Figs. 1 & 6–9) or condensed as a curved mass within the sporocyst cavity (Figs. 2–5). The ability of sporocyst content to take a curved condensed mass may represent a distinctive feature for this species. The sporulated oocysts (n = 30) range in shape from subspherical to spherical measuring 22.29–35.74 (30.76 ± 3.53) × 20.91–32.89 (28.13 ± 2.95) with shape index (length/width) of 1.02–1.18 (1.09 ± 0.05). Oocysts are predominantly subspherical (70%) than spherical (30%). The smallest oocyst (Fig. 6) is noticed to measure 22.29 × 20.91 and contains a micropyle-like structure that may represent a detachment or separation between outer and inner layers of the oocyst wall at this region. One ruptured oocyst is noticed measuring 44.30 × 32.63 with a shape index of 1.36 (Fig. 9).

The oocyst wall is double-layered, smooth, not striated or pitted, and measures 1.46–2.00 (1.65 ± 0.17) in thickness. The outer layer is thick and measures 0.82–1.20 (0.93 ± 0.11) while the inner layer is thinner and measures 0.60–0.90 (0.72 ± 0.09). In most cases, the oocyst wall shows a distinctive feature which is the presence of a lateral edge-like extension locating at one side of oocyst (Figs. 1, 4, 5, 8 & 9). In this region, the bilayered oocyst wall and lateral extension measure together 2.10–2.79 (2.51 ± 0.21). At top view, this extension or edge-like protrusion of oocyst wall is seen with a suture located in its middle (Fig. 6 *inset*). Oocyst rupturing and excystation appear to occur through the longitudinal axis of the suture in this edge-like extension (Figs. 8 & 9). Micropyle and oocyst residuum is not observed. Typically, one polar granule measuring 1.21–1.81 (1.52 ± 0.20) in diameter is noticed usually within the concavity of oocyst (Fig. 7) and rarely adhering to the inner surface of oocyst wall (Fig. 8). In rare cases, two small polar granules, each measures 0.82–1.12 (0.95 ± 0.11) in diameter, are seen within the oocyst cavity (Fig. 4).

II- Description of sporocysts:

The sporocysts are ovoid to ellipsoidal measuring 17.17–22.76 (20.89 ± 1.37) × 12.86–16.76 (15.04 ± 0.93) with shape index of 1.29–1.54 (1.39 ± 0.06). Stieda body is knob-like or rectangular in shape (Figs. 1–5, 7–9) measuring 1.23–2.35 (1.69 ± 0.35) high × 2.87–3.99 (3.30 ± 0.33) wide. Sub-Stieda body is irregularly rounded (Fig. 1), subglobular (Fig. 2), or rounded (Fig. 4) in shape measuring 2.23–2.89 (2.59 ± 0.23) high × 3.45–4.80 (4.40 ± 0.56) wide. Sporocyst residuum is a compact mass of small granules lying centrally among sporozoites (Fig. 7) and measures 4.05–5.31 (4.63 ± 0.45) × 2.57– 4.22 (3.14 ± 0.6). Sporocyst wall is single-layered, smooth, and measures 0.59–0.99 (0.75 ± 0.11). The

sporozoites are banana-shaped measuring 5.69–8.59 (7.3 ± 0.98) × 2.08–3.29 (2.80 ± 0.42) with shape index of 2.50–3.15 (2.74 ± 0.23).

B- Endogenous Stages (Figs. 10–18):

I- Site of Infection:

Endogenous stages of the present parasite occur usually in the enterocytes and to a lesser extent in *lamina propria* of the small intestine of their naturally-infected lizards. Developmental stages mostly took an apical position in regard to the nucleus of enterocytes.

II- Meronts and merozoites:

The uninucleate meronts (n = 10) measure 2.26–5.08 (3.68 ± 0.86) × 2.05–4.53 (3.05 ± 0.67), locate peripherally (Fig. 10) or centrally (Fig. 10 *inset*) within their large parasitophorous vacuoles and contain large nuclear material locating in central or peripheral of their bodies. These stages range in shape from spheroidal to ellipsoidal with a shape index of 1.02–1.63 (1.21 ± 0.17). Binucleate meronts (n = 3) measure 4.48–4.96 (4.78 ± 0.26) × 3.94–4.31 (4.06 ± 0.17) and locate centrally in the parasitophorous vacuole (Fig. 11). These stages are subspherical to ovoid with a shape index of 1.14–1.24 (1.18 ± 0.05).

Tri- and tetra-nucleate meronts (n = 3) are also noticed and measure 4.33–6.21 (5.38 ± 0.79) × 3.85–4.65 (4.31 ± 0.34) and 5.06–8.18 (6.62 ± 1.56) × 3.81–5.53 (4.67 ± 0.86), respectively. Trinucleate meronts (Fig. 12) are subspherical to ellipsoidal with a shape index of 1.13–1.34 (1.24 ± 0.09) while tetranucleate meronts (Fig. 13) are ellipsoidal with shape index of 1.33–1.48 (1.40 ± 0.07). Some multinucleate meronts are seen during the process of merozoites formation (Fig. 14). Multinucleate meronts contain up to 12 nuclei (Fig. 14 *inset*). Multinucleate meronts with more than 4 nuclei (n = 20) are spheroidal to ellipsoidal measuring 3.95–7.60 (6.49 ± 1.00) × 3.37–7.24 (5.35 ± 1.31) with shape index of 1.01–1.67 (1.26 ± 0.27). Merozoites are formed in meront with the formation of the residual body (Figs. 15–17). The number of merozoites formed per each meront extends from 3 (Fig. 15) to 11 (Fig. 17). Merozoites are usually arranged in a rosette pattern around a residual body in the center of meront (Figs. 16 & 17). The merozoites (n = 30) are banana-shaped measuring 2.51–3.29 (2.92 ± 0.27) × 0.72–1.20 (0.89 ± 0.17) with shape index of 2.73–3.68 (3.36 ± 0.34).

III- Microgamonts:

The microgamont (n = 1) is subspherical (Fig. 18) measuring 9.53 × 8.42 with a shape index of 1.13 and locates in a clear parasitophorous vacuole. Parasitophorous vacuoles are seen in most cases clearly surrounding meronts and/or gamonts. These vacuoles (N= 36) are spherical to ellipsoidal measuring 4.09–14.52 (9.42 ± 2.97) × 3.21–12.19 (7.79 ± 2.34) with shape index of 1.00–1.62 (1.21 ± 0.15).
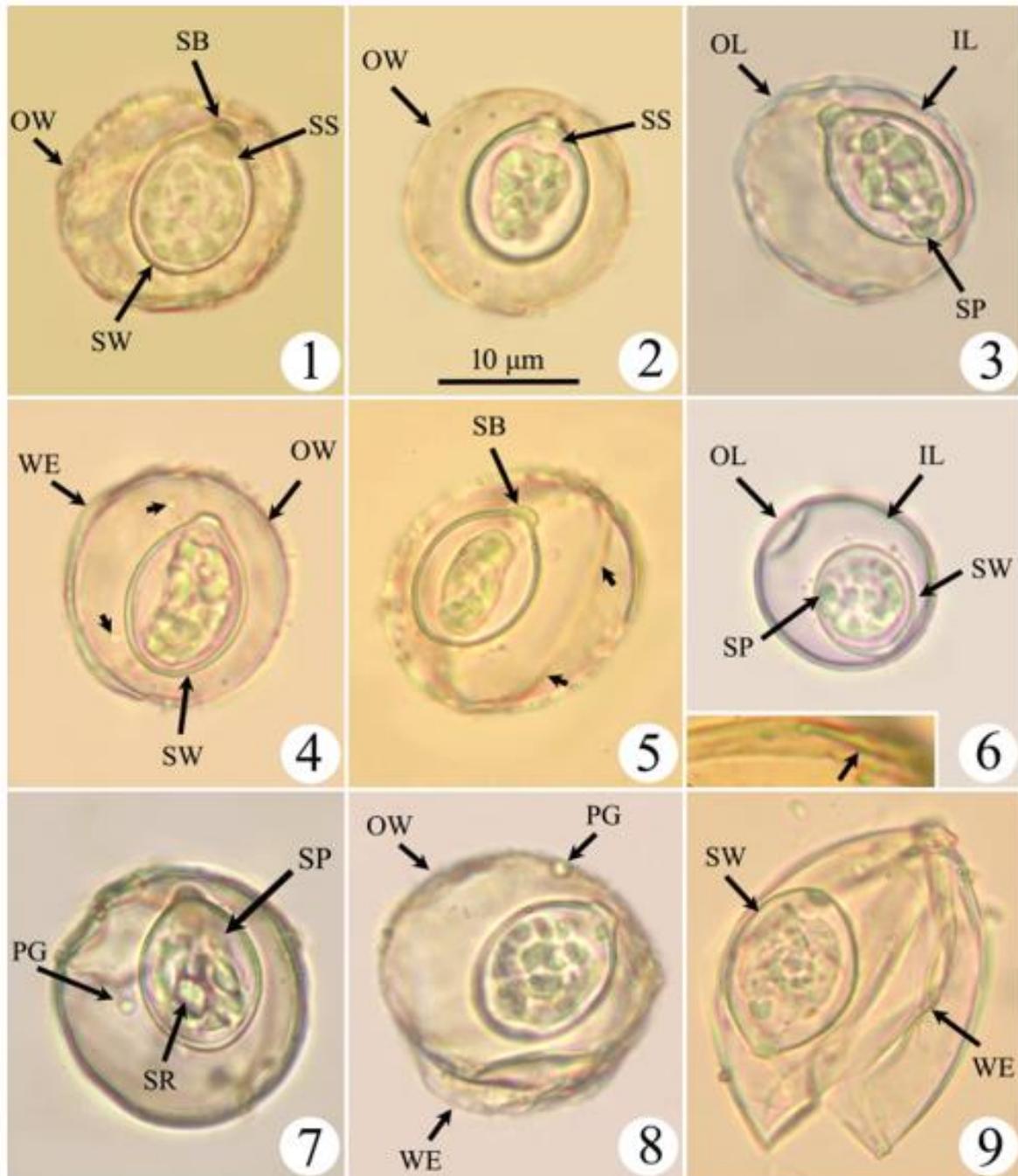
Figs. 1–8: Photomicrographs of sporulated oocysts of the present *Caryospora* species. (**1**) Oocyst containing a sporocyst with knob-like Stieda, irregular rounded sub-Stieda body and sporozoites filled sporocyst cavity. (**2**) Oocyst containing a sporocyst with subglobular sub-Stieda body. (**3–5**) Sporozoites form condensed curved masses within the sporocysts cavity. (**4**) Oocyst shows edge-like extension at one side of oocyst wall and two polar granules (short arrows). (**5**) Oocyst shows edge-like extension (short arrows). (**6**) Top view of the smallest recorded oocyst. (**6** *inset*) Top view of the oocyst wall edge-like extension and its suture (arrow). (**7**) Oocyst shows one polar granule and a compact sporocyst residuum. (**8**) Polar granule adhered to oocyst wall which opened at suture of edge-like extension. (**9**) Oocyst ruptured along the edge-like extension of oocyst wall. **Abbreviations:** IL, inner layer of oocyst wall; OL, Outer layer of oocyst wall; OW, oocyst wall; PG, polar granule; S, sporocyst; SB, Stieda body; SP, sporozoite; SR, sporocyst residuum; SS, sub-Stieda body; SW, sporocyst wall; WE, edge-like extension of oocyst wall. **Scale bar = 10 μm**
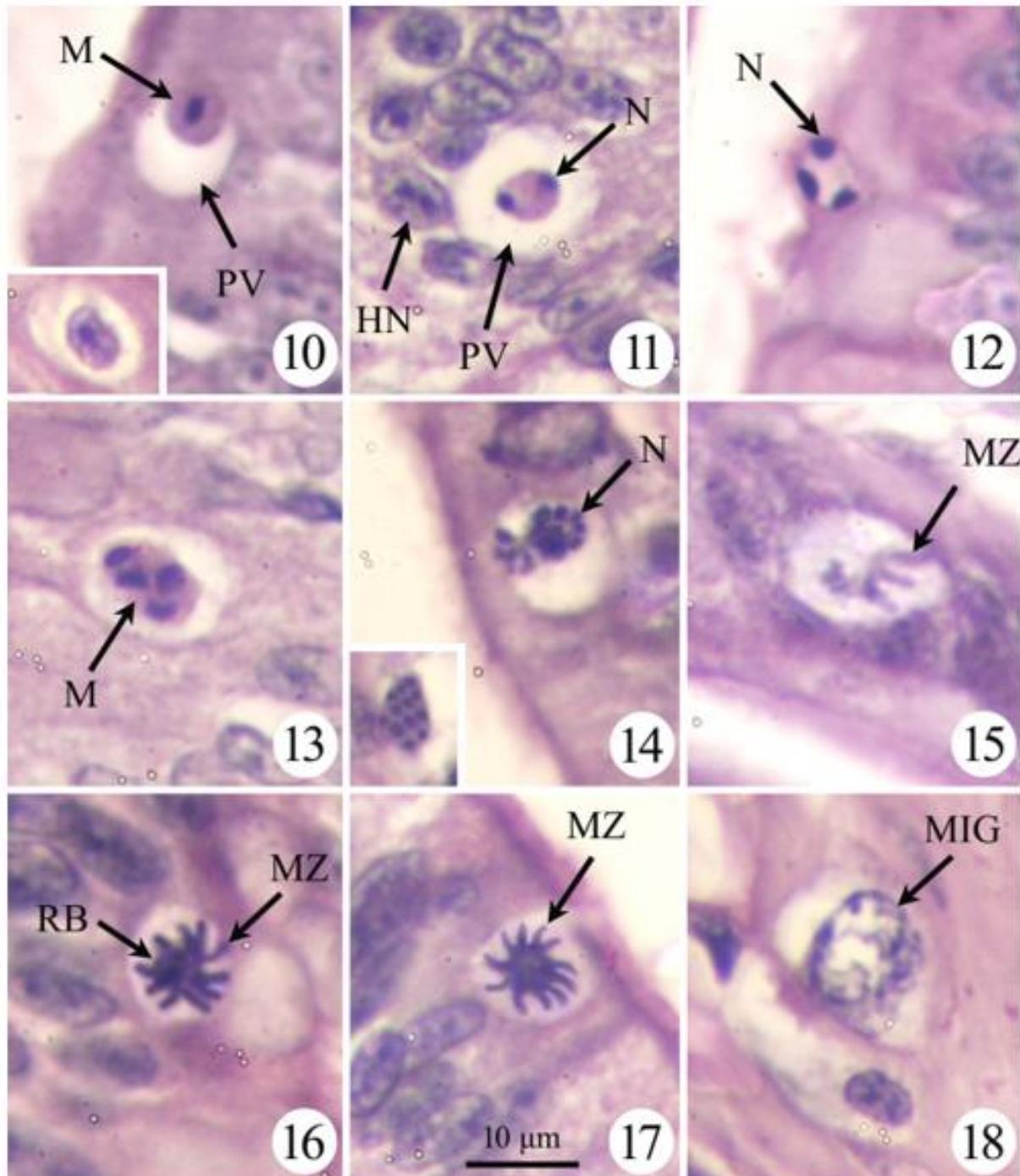
Figs. 10-18: Photomicrographs of endogenous stages of the present *Caryospora* species. **(10)** Uninucleate meront locates peripherally in parasitophorous vacuole adhering to its membrane. **(10 inset)** Uninucleate stage locates centrally in parasitophorous vacuole. **(11)** Binucleate meront. **(12)** Trinucleate meront. **(13)** Quadrinucleate meront. **(14)** Multinucleate meront in process of merozoites formation. **(14 inset)** Ellipsoidal multinucleate meront with 12 nuclei. **(15)** Meronts with 3 merozoites. **(16)** Meront with 10 merozoites arranged in a rosette-pattern around central residual body. **(17)** Meront with 11 merozoites arranged in a rosette-pattern; **(18)** Microgamont at process of microgametogenesis. **Abbreviations:** HN, host cell nucleus; M, meront; MIG, microgamont; MZ, merozoites; N, nucleus of parasite; PV, parasitophorous vacuoles; RB, residual body. **Scale bar = 10 μm**.

The sporulated oocysts of the present parasite were compared with other saurian *Caryospora* spp. reviewed by Upton *et al.* (1986) and compiled afterwards (Table 1). The overall prevalence of the present study was recorded of ∽ 12% (12/103) from the naturally infected agamid lizards. Different rates of natural infection were recorded for many caryosporan species, for example, 100% (2/2) in long-nosed vine snake *Ahaeiulta nusuia* infected by *C. ahaetullae* (Modrý and Koudela, 1994) and ornate Nile monitor, *Varanus ornatus* infected with *C. varaniornati* (Modrý *et al.*, 2001). In addition, the prevalence was reported of ∽ 36% (4/11) and ∽ 6% (1/18) in green lizard *Anolis carolinensis* infected by *C. ernsti* (Upton *et al.*, 1984a) and *C. natchitochesensis* (McAllister *et al.*, 2014), respectively. Moreover, the prevalence was recorded of ∽ 33% (1/3) in Eastern coachwhip *Masticophis f. flagellum* infected with *C. masticophis* (Upton *et al.*, 1994), 25% (1/4) in horned bush-viper *Atberis ceratopborus* infected by *C. matatu* (Modrý *et al.*, 2002) and 75% (3/4) in Guatemalan snake *Conophis lineatus* infected by *C. conophae* (Seville *et al.*, 2005).

Sporulation of the present parasite is endogenous with oocyst passed sporulated in host feces. Similar observations were noticed in a few reptilian *Caryospora* spp. such as *C. demansiae* (Cannon, 1967) and *C. natchitochesensis* (McAllister *et al.*, 2014) infecting snake and lizard hosts, respectively. However, many other species had exogenous sporulation with different sporulation times, for example, *C. ernsti* (Upton *et al.*, 1984a), *C. varaniornati* (Modrý *et al.*, 2001) infecting saurian hosts and *C. cobrae* (Nandi, 1985), *C. telescopis* (Matuschka, 1986b), *C. epicratesi* (Lainson *et al.*, 1991), *C. ahaetullae* (Modrý and Koudela, 1994), *C. matatu* (Modrý *et al.*, 2002), *C. choctawensis* (McAllister *et al.*, 2012) infecting Serpentes.

The typical body shape of the sporulated oocysts for most recorded *Caryospora* species were spherical and/or subspherical (Upton *et al.*, 1986). The sporulated oocysts of the present parasite were predominantly subspherical (70%) to spherical (30%) with shape index 1.02–1.18 (1.09). These results were in full agreement also with oocysts of *C. pseustesi*, *C. epicratesi C. carajasensis,* and *C. constanciae* that predominantly subspherical with values of 60%, 82%, 87%, and 88%, respectively (Lainson *et al.*, 1991). In contrary, other species such as *C. micruri*, *C. paraensis* (Lainson *et al.*, 1991) and *C. matatu* (Modrý *et al.*, 2002) were recorded predominantly spherical than subspherical while many other species were reported to have only spherical or nearly rounded oocysts such as *C. gekkonis* (Chakravarty and Kar, 1947)**,** *C. ernsti* (Upton *et al.*, 1984a), *C. najadae* (Matuschka, 1986a), *C. telescopis* (Matuschka, 1986b), *C. tantillae*, *C. relictae* (Telford, 1997) and *C. veselyi* (Modrý and Koudela, 1998). Few species were recorded to have other forms deviated from the previous typical body plan of oocyst shape, for example, *C. natchitochesensis* had subspheroidal to ovoidal shape (McAllister *et al.*, 2014). In addition, *C. cheloniae* (Leibovitz *et al.*, 1978) and *C. schokariensis* (Alyousif *et al.*, 2004) were unique in having elongated ellipsoidal oocysts.

The sporulated oocyst of the present species measured 22.29–35.74 × 20.91–32.89 (30.76 × 28.13) and this showed a convergence of measurements to other species such as *C. najadae* (Matuschka, 1986a), *C. peruensis* (Upton *et al.*, 1989), *C. madagascariensis* (Upton *et al.*, 1990), *C. pseustesi*, *C. micruri* (Lainson *et al.*, 1991), *C. gracilis* (Upton *et al.*, 1992b), *C. ahaetullae* (Modrý and Koudela, 1994), *C. guatemalensis***,** *C. mayorum* (Seville *et al.*, 2005) and *C. olfersii* (Viana *et al.*, 2013). However, the sporulated oocysts of the present parasites were smaller than *C.*

maxima (Modrý *et al.*, 1999) infecting a snake host but larger than all known *Caryospora* spp. reported from the lacertilian hosts (Chakravarty and Kar, 1947; Upton *et al.*, 1984a; Modrý *et al.*, 2001; McAllister *et al.*, 2014) and *C. cobrae* (Nandi, 1985), *C. serpentis* (Upton *et al.*, 1990), *C. micruri*, *C. paraensis* (Lainson *et al.*, 1991), *C. maculatus* (Upton *et al.*, 1992a), *C. masticophis* (Upton *et al.*, 1994), *C. kalimantanensis* (Modrý and Koudela, 1997), *C. veselyi* (Modrý and Koudela, 1998), *C. matatu* (Modrý *et al.*, 2002), *C. bothriechis* (Seville *et al.*, 2005), *C. durrelli* (Daszak *et al.*, 2011) and *C. choctawensis* (McAllister *et al.*, 2012) infecting snakes.

The oocyst wall of the present species is bilayered, not pitted or striated, and has a smooth surface. Similar observations were noticed in many other *Caryospora* spp. such as *C. gekkonis* (Chakravarty and Kar, 1947)**,** *C. natchitochesensis* (McAllister *et al.*, 2014) infecting lizards and *C. cobrae* (Nandi, 1985), *C. carajasensis*, *C. pseustesi*, *C. epicratesi*, *C. constanciae* (Lainson *et al.*, 1991), *C. kalimantanensis* (Modrý and Koudela, 1997), *C. veselyi* (Modrý and Koudela, 1998), *C. maxima* (Modrý *et al.*, 1999), *C. regentensis* (Daszak and Ball, 2001), *C. matatu* (Modrý *et al.*, 2002), *C. bothriechis*, *C. coniophanis*, *C. conophae*, *C. guatemalensis*, *C. mayorum* and *C. zacapensis* (Seville *et al.*, 2005) infecting snakes. However, some species have unilayered oocyst wall such as *C. ernsti* (Upton *et al.*, 1984a), *C. varaniornati* (Modrý *et al.*, 2001) infecting saurian hosts and *C. micruri*, *C. paraensis* (Lainson *et al.*, 1991) and *C. ahaetullae* (Modrý and Koudela, 1994) infecting Serpentes. In contrary, *C. brasiliensis* (Lainson and Shaw, 1973) and *C. olfersii* (Viana *et al.*, 2013) have trilayered oocyst wall. Moreover, striated or pitted oocyst wall was seen in many *Caryospora* species such as *C. ernsti* (Upton *et al.*, 1984a), *C. peruensis* (Upton *et al.*, 1989), *C. barnardae*, *C. brygooi*, *C. serpentis* (Upton

et al., 1990), *C. carajasensis*, *C. epicratesi*, *C. pseustesi* (Lainson *et al.*, 1991), *C. heterodermus* (Upton *et al.*, 1992a), *C. gracilis* (Upton *et al.*, 1992b), *C. kalimantanensis* (Modrý and Koudela, 1997), *C. veselyi* (Modrý and Koudela, 1998), *C. matatu* (Modrý *et al.*, 2002), *C. conophae*, *C. coniophanis*, *C. guatemalensis*, *C. mayorum*, *C. zacapensis* (Seville *et al.*, 2005), *C. olfersii* (Viana *et al.*, 2013) and *C. ceadsensis* (de Santana Miglionico and Viana, 2017). The present parasite shows a distinctive characteristic feature which is the presence of a sutured edge-like extension located on side of oocyst wall and this feature wan not found in any other reptilian *Caryospora* species.

Oocyst residuum and micropyle were absent in oocysts of the present caryosporan species. The present results were reinforced with the majority of *Caryospora* species such as *C. ernsti* (Upton *et al.*, 1984a)**,** *C. varaniornati* (Modrý *et al.*, 2001)**,** *C. ahaetullae* (Modrý and Koudela, 1994), *C. maxima* (Modrý *et al.*, 1999), *C. bothriechis*, *C. coniophanis*, *C. conophae*, *C. guatemalensis*, *C. mayorum*, *C. zacapensis* (Seville *et al.*, 2005), *C. durrelli* (Daszak *et al.*, 2011) and *C. ceadsensis* (de Santana Miglionico and Viana, 2017). However, micropyle was present in four *Caryospora* spp. of reptiles, namely, *C. legeri* (Hoare, 1933), *C. gekkonis* (Chakravarty and Kar, 1947), *C. bengalensis* (Mandal, 1976) and *C. cobrae* (Nandi, 1985). In addition, oocyst residuum was present in *C. heterodermus* (Upton *et al.*, 1992a), *C. veselyi* (Modrý and Koudela, 1998), and *C. matatu* (Modrý *et al.*, 2002). Moreover, oocyst residuum is formed of one to several masses of debris in *C. gracilis* (Upton *et al.*, 1992b).

The obtained result revealed that the present parasite has one or rarely two rounded polar granules that lying in the oocyst cavity or adhered to the inner surface of the oocyst wall. Similar observations were noticed in *C. ernsti* (Upton *et al.*, 1984a), *C. ahaetullae* (Modrý and Koudela, 1994), *C. bothriechis*, *C.*

coniophanis, *C. conophae*, *C. guatemalensis*, *C. mayorum*, *C. zacapensis* (Seville *et al.*, 2005) and *C. ceadsensis* (de Santana Miglionico and Viana, 2017). In addition, *C. epicratesi* (Lainson *et al.*, 1991) has a single polar granule adhered to the inner layer of oocyst wall (36%) or sporocyst wall (67%). Moreover, *C. choctawensis* (McAllister *et al.*, 2012)

possesses a single bilobed polar granule. However, absence of polar granule was noticed in *C. cobrae* (Nandi, 1985), *C. carajasensis*, *C. pseustesi* (Lainson *et al.*, 1991), *C. tantillae* (Telford, 1997), *C. maxima* (Modrý *et al.*, 1999), *C. schokariensis* (Alyousif *et al.*, 2004), *C. durrelli* (Daszak *et al.*, 2011) and *C. olfersii* (Viana *et al.*, 2013).

Table 1: Comparison of morphometric descriptions and measurements of the present *Caryospora* sp. and other *Caryospora* spp. recorded from saurian hosts.

Species (Reference)	– Type host – Type locality – Prevalence – Sporulation	Sporulated oocysts – Shape, size "L × W" – Shape index "L / W ratio" – Wall layer (s), TT; features – PG (s) number &/or size – Micropyle – Oocyst residuum	Sporocysts – Shape, size "L × W" – Shape index "L / W ratio" – Wall layer (s), TT or features – SB shape &/or size "H × W" – SSB shape &/or size "H × W" – SP shape &/or size "L × W" – SR dispersal &/or size
C. gekkonis (Chakravarty and Kar, 1947)	– Tokay gecko *Gekko gecko* – Calcutta, India – No data – Exogenous	– Spherical, 19.8 – No data – Bilayered, no data; IL thicker – Absent – Present – Absent	– Spherical, 11.0 – No data – No data – Present – No data – No data – Diffuse
C. ernsti (Upton *et al.*, 1984a)	– Green lizard *Anolis carolinensis* – Southeastern USA – ⌣36% (4/11) – Exogenous	– Spherical, 11.0–14.5 (12.5) – No data – Unilayered, *c.* 1.0; OL lightly pitted, IL smooth – Single (AIL), 1.0–1.5 (1.3), occasionally, 1 or 2 additional non-refractile granules (AIL) present, *c.* 1.0 – Absent – Absent	– Ovoid, 10–12.5 × 7.5–9 (10.7 × 8.3) – 1.2–1.4 (1.3) – Unilayered, *c.* 0.7, smooth – Nipple-like*, 1.8–3.0 × 0.5–1.0 (2.2 × 0.7) – Rounded*, 2.5–4.2 × 0.8–1.5 (3.2 × 1.1) – Elongate, 8–11 × 2–2.2 (9.7 × 2.0) – Compact*, 3.6–6.3 × 2.7–5.4 (5.6 × 3.8)
C. varaniornati (Modrý *et al.*, 2001)	– Ornate Nile monitor, *Varanus ornatus* – Benin, Africa – 100% (2/2) – Exogenous	– Spherical to slightly subspherical, 11–12.5 × 11–12 (12.0 × 11.5) – 1.0–1.09 (1.04) – Unilayered, 0.75; smooth – Single (AIL*), *c.* 1.0–1.5 – Absent – Absent	– Broadly ellipsoidal, 8.5–9.5 × 6.5–7 (8.8 × 6.7) – 1.2–1.5 (1.32) – Unilayered, smooth – Lentil-like, *c.* 0.5 × 1.0 – Absent – Elongate – Diffuse
C. natchitochesensis (McAllister *et al.*, 2014)	– Green lizard *Anolis carolinensis* – Louisiana, USA – ⌣ 6% (1/18) – Endogenous	– Subspheroidal to ovoidal, 11–15 × 10–14 (13.1 × 12.3) – 1.0–1.2 (1.1) – Bilayered, *c.* 0.3–0.7; OL & IL equal – Single (AIL*) – Absent – Absent	– Ovoidal, 7–13 × 6–10 (10.1×7.4) – 1.1–1.5 (1.4) – Unilayered, smooth – Flattened – Absent – Sausage-shaped – Compact or diffuse
C. savignii (present species)	– Savigny's Agama *Trapelus savignii* – Eastern Desert, Egypt – ⌣ 12 (12/103) – Endogenous	– Subspherical (70%) to spherical (30%), 22.29–35.74 × 20.91–32.89 (30.76 × 28.13) – 1.02–1.18 (1.09) – Bilayered, 1.46–2.00 (1.65); smooth, OL, 0.82–1.20 (0.93), IL, 0.60–0.90 (0.72) – Single (IC or AIL), rarely double – Absent – Absent	– Ovoid to ellipsoidal, 17.17–22.76 × 12.86–16.76 (20.89 × 15.04) – 1.29–1.54 (1.39) – Unilayered, 0.59–0.99 (0.75), smooth – Knob-like or rectangular, 1.23–2.35 × 2.87–3.99 (1.69 × 3.30) – Rounded, irregular rounded or subglobular, 2.23–2.89 × 3.45–4.80 (2.59 × 4.40) – Sausage-shaped, 5.69–8.59 × 2.08–3.29 (7.3 × 2.80) – Compact, 4.05–5.31 × 2.57–4.22 (4.63 × 3.14)

Abbreviations; AIL, adhered to the inner layer of oocyst wall; *c.*, *circa*; H, high; IC, inside oocyst cavity not adhered to oocyst or sporocyst wall; IL, the inner layer of oocyst wall; L, length; OL, the outer layer of oocyst wall; PG (s), polar granule (s); SB, Stieda body; SP, sporozoite; SR, sporocyst residuum; SSB, sub-Stieda body; TT, total thickness; W, width (wide); *, not described by original authors but described by present author from original papers. Description of SB & SSB followed Berto *et al.* (2014). All measurements are in micrometres (µm) and presented as the range followed by the mean values in parentheses.

Sporocysts of the present parasite were ovoid to ellipsoidal measuring 17.17–22.76 × 12.86–16.76 (20.89 × 15.04) with shape index of 1.29–1.54 (1.39). Different measurements and shape indices of sporocysts of saurian *Caryospora* spp. were compiled in Table (1). Sporocysts of the present species have smooth, unilayered wall, Stieda, sub-Stieda bodies, and a compact sporocyst residuum. Similar observations were noticed for many caryosporan species such as *C. cheloniae* (Leibovitz *et al.*, 1978), *C. najadae* (Matuschka, 1986a), *C. heterodermus* (Upton *et al.*, 1992a), *C. tantillae*, *C. relictae* (Telford, 1997), *C. regentensis* (Daszak and Ball, 2001) and *C. matatu* (Modrý *et al.*, 2002). However, many differences were reported in other species such as the presence of bilayered sporocyst wall in *C. cobrae* (Nandi, 1985), the absence of sub-Stieda body in *C. cobrae* (Nandi, 1985), *C. tantillae* (Telford, 1997), *C. varaniornati* (Modrý *et al.*, 2001), *C. bothriechis* (Seville *et al.*, 2005), *C. natchitochesensis* (McAllister *et al.*, 2014) and the presence of diffuse sporocyst residuum in *C. masticophis* (Upton *et al.*, 1994), *C. varaniornati* (Modrý *et al.*, 2001) and *C. ceadsensis* (de Santana Miglionico and Viana, 2017).

Morphometric descriptions and measurements of merogonic stages of the present species were compared with those recorded from other reptilian *Caryospora* spp. infecting their primary hosts (Table 2). Endogenous stages of the present parasite locate frequently in the cytoplasm of the enterocytes and to a lesser extent in *lamina propria* of the small intestine of the infected hosts occupying a mainly apical position in regard to intestinal epithelia. Infection isn't found in any other organ. Similar observations were recorded for many other *Caryospora* spp. and small intestine appeared to be the most common site of infection in primary hosts as recorded for *C. demansiae* (Cannon, 1967), *C. dendrelaphis* (Cannon and Rzepczyk, 1974), *C. bigenetica* (Wacha and Christiansen, 1982) and *C.*

colubris (Paperna and Finkelman, 1991). In addition, endogenous stages were recorded in hindgut epithelium (Leibovitz *et al.*, 1978), brain and epithelium, and/or *lamina propria* of the crop or intestine (Chapman *et al.*, 2016) of green sea turtles *Chelonia mydas* infected by *C. cheloniae*. Caryocysts aren't formed in primary host tissues (Wacha and Christiansen, 1982) as recorded for the present parasite. However, endogenous stages including the formation of different meronts generations, gamonts, gametes, zygotes, membrane-bound sporulated oocysts, sporozoites liberation from oocysts and infection of other cells forming caryocysts with up to 4 sporozoites were noticed in fibroblast-like cells of the connective tissues of the nose, cheek, scrotum and less frequently tongue of experimental mice as secondary hosts and different tissue culture cell lines (Upton *et al.*, 1984b; Upton and Barnard, 1988; Sundermann *et al.*, 1988).

Meront shapes of the present study showed some degrees of similarities with other recorded caryosporan species but differed in measurements (Table 2). Meronts of the parasite under investigation yielded a low number of merozoites (3–11) arranged in a rosette-like pattern around the residual body in the center of meront. The low number of merozoites was also recorded in 1st and 2nd generation meronts of *C. bigenetica* yielding 10–14 (12), 4–9 (7), respectively, inside the snake host (Wacha and Christiansen, 1982)**.** However, Upton and Barnard (1988) recorded 8–12 (10)**,** 22–28 (24), and 12–18 (16) for 1st, $2^{nd,}$ and 3rd generation meronts, respectively, in extraintestinal tissues of mice experimentally infected by *C. bigenetica*. Merozoites formed without leaving any residual body were recorded in 1st and 2nd meronts of *C. bigenetica* (Wacha and Christiansen, 1982) and meronts of *C. colubris* (Paperna and Finkelman, 1991) inside their snake hosts. However, residuum was observed in meronts of *C. simplex* (Upton *et al.*, 1984c), type I–III

meronts of *C. bigenetica* (Upton and Barnard, 1988) and type I & II meronts of *C. bigenetica* (Sundermann *et al.*, 1988).

According to the number of meronts per each host cell, only one meront was observed, usually within a clear parasitophorous vacuole, in the same enterocyte of the present study. Similar observations were noticed for most *Caryospora* species (Wacha and Christiansen, 1982; Paperna and Finkelman, 1991). However, Upton and Barnard (1988) noticed up to three meronts III (but usually one) were seen in the same host cell and each was located within a spherical or ovoid parasitophorous vacuole. Microgamont is the only gamogonic stage observed in the present study. It is subspherical measuring 9.53 × 8.42. Microgamonts of various *Caryospora* species were recorded with different measurements (Paperna and Finkelman, 1991; Sundermann *et al.*, 1988).

Table 2: Comparison of morphometric descriptions and measurements of merogonic stages of the present *Caryospora* sp. and some other *Caryospora* spp. inside their primary hosts.

Species (References)	– Location of merogonic stages	Merogony – Uninucleate meront shape &/or size – Multinucleate meront shape &/or size – Merozoites number – Merozoites shape &/or size
C. demansiae (Cannon, 1967)	– Small intestine epithelium	– *c.* 4 – 6–12 – 4, 8, 16 – 3–5 x 1.5
C. dendrelaphis (Cannon and Rzepczyk, 1974)	– Small intestine epithelium	– 3–6 – 5–9 – 16, 32, 64 – 1.5 x 1
C. cheloniae (Leibovitz *et al.*, 1978; Chapman *et al.*, 2016)	– Hindgut epithelium, brain, epithelium and/or *lamina propria* of crop or intestine	– Cigar-shaped, 9.2–10.7 × 7.9–8.6 (10.3 × 8.4) – 30–80 – No data – ≤ 1
C. bigenetica (Wacha and Christiansen, 1982)	– Intestinal epithelium	**1ˢᵗ generation meront** – No data – Subspherical to ellipsoid, 8.3–15.4 × 5.1–10.9 (11.7 × 8.0) – 10–14 (12) – Lanceolate, 3.8–5.1 × 1.3–1.6 (4.7 × 1.3) **2ⁿᵈ generation meront** – No data – Spherical to ellipsoid, 6.4–12.8 × 5.1–8.3 (9.7 × 6.3) – 4–9 (7) – Lanceolate, 3.8–5.8 × 1.0–1.9 (5.2 × 1.5)
C. colubris (Paperna and Finkelman, 1991)	– 3rd quarter of the intestine with a few occurring in the 2nd quarter	– 4.1–8.1 × 2–2.4 – 8–13 × 7–10 (developing meront, 6–8 × 5–6) – 8 – 7–8 × 1.2–2.0
C. savignii (present species)	– Small intestine epithelium	– Spheroidal to ellipsoidal, 2.26–5.08 × 2.05–4.53 (3.68 × 3.05) – Spheroidal to ellipsoidal, 3.95–7.60 × 3.37–7.24 (6.49 × 5.35) – 3–11 – Banana-shaped, 2.51–3.29 × 0.72–1.20 (2.92 × 0.89)

Abbreviations; c., *circa*. All measurements are in micrometres (μm) and presented as the range followed by the mean values in parentheses.

The present parasite is described for the first time from the agamid lizards *Trapelus savignii* from the Eastern Desert in Egypt. Due to the large size of its sporulated oocysts as compared with other *Caryospora* spp. recorded from saurian hosts and the existence of some distinctive features which include the presence of a lateral sutured edge-like extension of oocyst wall and the ability of sporocyst contents to form a curved condensed mass within the sporocyst cavity, the current parasite is suggested to be a new species and named *C. savignii* taking the specific name of its type-host.

Taxonomic summary:

Caryospora savignii n. sp.

Type-host: The agamid lizard *Trapelus savignii* Duméril and Bibron, 1837.

Type-locality: Eastern Desert, Egypt.

Type-specimens: Sporulated oocysts kept in 10% formalin, slides, phototypes, and line drawing are deposited in the parasitological collection of Zoology & Entomology Department, Faculty of Science, Helwan University, Egypt.

Prevalence: 11.65% (12/103).

Sporulation: Endogenous.

Site of endogenous stages: Meronts and microgamonts occur mainly in enterocytes of the small intestine. Merogony yielded 3-11 uninucleate merozoites.

Etymology: The suggested specific epithet of the present parasite is derived from the name of type-host species.

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ARABIC SUMMARY

دراسة بالمجهر الضوئى لنوع كاريوسبورا (معقدات القمة: أيميريدى) من قاضى الجبل الرملى
"*Trapelus savignii*" (زواحف: حرذونيات) فى مصر

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تم فى هذا البحث وصف مراحل النمو لنوع جديد مُقَترح من الكاريوسبورا ليجر 1904 من سحلية قاضى الجبل الرملى "*Trapelus savignii*" التى تم تجميعها خلال صيف 2019 من الصحراء الشرقية بمصر حيث بلغ معدل الإصابة الكُلى 11.65% (103/12) وتم التجرثم داخلى وتتراوح الأكياس البيضية المتجرثمة بأشكالها من شبه الكروية (70%) إلى الكروية (30%) ويبلغ قياساتها 22.29–35.74 × 20.91–32.89 ميكرون (الطول × العرض). ويأتى جدار الكيس البيضى ثنائى الطبقات ويبلغ قياسه 1.46–2.00 ميكرون. وتشمل الصفات المميزة لمعظم الأكياس البيضية وجود إمتداد محزوز يشبه الحافة على أحد جانبى جدار الكيس البيضى وكذلك قدرة محتوى الكيس الجرثومى على التشكل لكتلة منحنية مكثفة داخل تجويف الكيس الجرثومى. وعادة ما توجد واحدة أو أثنتان من الحبيبات القطبية كما تميزت الأكياس البيضية بغياب الفويهة وبقايا الكيس البيضى. وتأتى الأكياس الجرثومية بيضاوية الشكل مسجلةً 17.17–22.76 × 12.86– 16.76 ميكرون كما تم تعيين السمات الشكلية وقياسات جسم ستيدا وتحت ستيدا وبقايا الأكياس الجرثومية. وجاءت السبوروزويتات موزية الشكل مسجلةً قياساً 5.69–8.59 × 2.08–3.29. وتسكن مراحل الميروجنى والميكروجامونتات (أمهات الأمشاج المذكرة) الخلايا الطلائية المبطنة للأمعاء الدقيقة بشكل أساسى حيث توجد فى معظم الأحيان بداخل فجوات طفيلية واضحة. وتقيس الميرونتات الناضجة 3.95–7.60 × 3.37–7.24 ميكرون مكونة من 3–11 من الميروزويتات مع تكوين بقايا الميرونتات. والميروزويتات موزية الشكل وتقيس 2.51–3.29 × 0.72–1.2 ميكرون. وقد لوحظ أن الميكروجامونت يقيس 9.53 × 8.42 ميكرون.