

Caryospora varaniornati sp. n. (Apicomplexa: Eimeriidae) in the Nile monitor, *Varanus (Polydaedalus) niloticus* species complex

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Abstract. Parasitological examination of two ornate Nile monitors *Varanus ornatus* (Daudin, 1803) imported from Benin revealed the presence of a new species of *Caryospora*. Oocysts of *Caryospora varaniornati* sp. n. are spherical to slightly subspherical, $12.0 (11-12.5) \times 11.5 (11-12) \mu\text{m}$, without a micropyle and oocyst residuum, and occasionally possessing one small polar granule. Sporocysts are broadly ellipsoidal, $8.8 (8.5-9.5) \times 6.7 (6.5-7) \mu\text{m}$; a lentil-like Stieda body is present, ca. $0.5 \times 1 \mu\text{m}$; substieda body not visible. Experimental infection of a closely related host, *Varanus niloticus* (L.), did not lead to the oocyst excretion despite the fact that one of the experimentally inoculated monitors was immunosuppressed by dexamethasone. Histological examination did not reveal stages of coccidian development. Therefore, it is possible that *C. varaniornati* is strictly host specific.

Coccidia of the genus *Caryospora* Léger, 1904 (Apicomplexa: Eimeriidae) are widely distributed among snakes and raptorial birds and only a few species have been described from other hosts. Only two species have been described to date from saurian hosts in contrast to the diversity of these coccidia in snakes, from which 46 caryosporan species are listed (Modrý 1999). In this paper we describe a new *Caryospora* species from the African monitor lizard *Varanus ornatus* (Daudin, 1803) (Sauria: Varanidae), a member of the poorly understood *Varanus niloticus* (L.) species complex.

MATERIALS AND METHODS

An adult specimen of the ornate Nile monitor, *Varanus (Polydaedalus) ornatus* from ZOO Brno, at that time hospitalised at the University of Veterinary and Pharmaceutical Sciences, Brno due to a fracture of the hind limb, was parasitologically examined. Based on data provided by the owner (ZOO Brno), the animal originated from a group of wild-caught Nile monitors recently imported by a pet-trader from Benin in West Africa. The hospitalised animal was kept in quarantine in a glass terrarium and fed on day-old chicks and adult laboratory mice. Faecal samples were collected repeatedly from the floor of the cage and submitted for parasitological examination. Later, faecal samples of a cage-mate ornate Nile monitor of the same origin were also provided for parasitological examination by ZOO staff. The

faecal samples were examined microscopically after concentration by flotation with modified Sheather's sugar solution (s.g. 1.30). Fresh samples containing unsporulated coccidian oocysts were allowed to sporulate in Petri dishes at room temperature (20-23°C) and examined daily to determine the stage of sporulation. Sporulated oocysts were measured and photographed using Nomarski interference contrast (NIC) optics. Measurements were made using a calibrated ocular micrometer and are reported in micrometres, as means, followed by the ranges in parentheses. Sporulated oocysts were purified by flotation in Sheather's sugar solution and stored in 2.5% aqueous solution (w/v) of potassium dichromate at 4°C for one month before further use.

Two juvenile, ca. 6 months old, *Varanus (Polydaedalus) niloticus* were used for experimental infection. Both animals originated from Togo and had been housed separately and were coprologically negative for previous two months. Two days prior the infection, one animal (No. 2) was given $9 \text{ mg} \cdot \text{kg}^{-1}$ of dexamethasone (DEXASON, ICN Galenica, Yugoslavia). Additionally, three severe combined immunodeficient (SCID) mice were used for experimental infection. SCID mice were maintained in an isolation room under pathogen-free conditions and kept as described previously (Vítvec et al. 1997). Immediately before inoculation, sporulated caryosporan oocysts were washed three times by centrifugation in tap water and then incubated for 15 min with 2.0% (v/v) peracetic acid to kill any adherent bacteria. The oocyst suspensions were then washed three times with sterile phosphate-buffered saline (PBS) and the number of oocysts present in each inoculum determined using a haemocytometer.

Both Nile monitors and SCID mice were inoculated orally with 10^3 sporulated oocysts. Faeces of both Nile monitors were collected irregularly, based on their feeding and defecating habits, and examined by sucrose flotation. SCID mice and Nile monitor treated previously by dexamethasone were euthanised and dissected at 30 days post infection (DPI) (mice) and 90 DPI (monitor). A total of 26 tissue samples (liver, pancreas, gall bladder, spleen, lung, kidney, urinary bladder, brain, tongue, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, eyelid, nose, foot pads, upper cheek, jowl, scrotum, diaphragm and gluteal, triceps brachii and cardiac muscles) from each SCID mouse were excised and fixed in 10% neutral buffered formalin; samples of gall bladder, liver, stomach, and 8 equidistantly spaced samples of the intestine were collected from the Nile monitor. Fixed tissues were processed for light microscopy using standard methods. Paraffin sections were stained with haematoxylin/eosin (HE) and Giemsa.

RESULTS

Coprological examination of both ornate Nile monitors, *Varanus ornatus*, originating from Benin revealed the presence of strongylid eggs and unsporulated coccidian oocysts in all the samples examined.

Both experimentally inoculated Nile monitor lizards *Varanus niloticus* remained coprologically negative for the consequent three months; histological examinations of one of them (No. 2) revealed no developmental stages of coccidia. Similarly, SCID mice did not develop typical dermal caryosporosis and histological examination of their tissues did not reveal any coccidian parasites.

The recovered coccidium evidently represents a new caryosporan species, which is described below.

Caryospora varaniornati sp. n.

Figs. 1-4

Oocysts monosporocystic, octozoic, spherical to slightly subspherical, $12.0 (11-12.5) \times 11.5 (11-12)$, with a shape index (SI) 1.04 (1.0-1.09); micropyle and oocyst residuum absent. A small (ca. 1-1.5) polar granule occasionally present. Oocyst wall smooth, colourless, single-layered, 0.75 thick. Sporocysts broadly ellipsoidal, $8.8 (8.5-9.5) \times 6.7 (6.5-7)$, with smooth, colourless and unilayered sporocyst wall; SI 1.32 (1.2-1.5). Stieda body present, lentil-like, ca. 0.5 high and 1 wide; substieda body not visible. Sporozoites elongate, each with subspherical refractile body and centrally located nucleus. Sporocyst residuum present, usually as small granules scattered among sporozoites.

Type host: Ornate Nile monitor, *Varanus (Polydaedalus) ornatus* (Daudin, 1803) (Sauria: Varanidae).

Type locality: Type-host individuals were imported from Benin, Africa; detailed locality unknown.

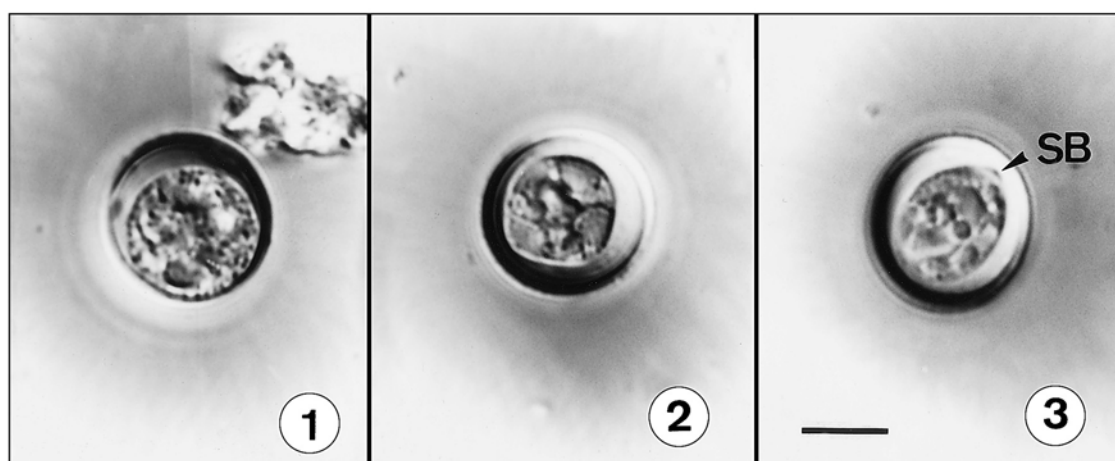
Type specimens: Phototypes are deposited in Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice; Coll. No. R 170/98.

Site of infection: Unknown.

Sporulation: Exogenous. First fully sporulated oocysts were observed at day 13.

Prevalence: 2/2 (100%).

Etiology: The species name *varaniornati* is made by joining the genitive forms of host generic and specific Latin names (*varanus*, -i, *ornatus*, -i).



Figs. 1-3. Nomarski interference contrast (NIC) photographs of the oocysts of *Caryospora varaniornati* sp. n., all in the same scale. **Fig. 1.** Unsporulated oocyst from fresh faeces. **Fig. 2.** Oocyst in advanced stage of sporulation, after 9 days. **Fig. 3.** Sporulated oocyst. SB – Stieda body. Scale bar = 5 μ m.

DISCUSSION

Caryosporan coccidia represent relatively common parasites of snakes and raptorial birds from which more than 70 species have been described to date (Upton et al. 1986, 1990, Modrý 1999, Volf et al. 2000). However, the presence of parasites of this genus in saurian hosts is exceptional. *Caryospora gekkonis* Chakravarty et Kar, 1947 from *Gekko gekko* (Sauria: Gekkonidae) in India and *Caryospora ernsti* Upton, Current et Barnard, 1984 from *Anolis carolinensis* (Sauria: Anolidae) in Northern to Southern America (Chakravarty and Kar 1947, Upton et al. 1984a) are the only species described from saurians. *Caryospora gekkonis* is easily distinguishable from *C. varaniornati* in having much larger oocysts with distinct micropyle. Although *C. ernsti* is similar in appearance to *C. varaniornati*, it differs in having slightly larger oocyst ($11-14.5$ vs. $11-12.5$) and sporocyst ($10-12.5 \times 7.5-9$ vs. $8.5-9.5 \times 6.5-7$) dimensions. Additionally, sporocysts of *C. ernsti* possess a distinct substieda body, which was not seen in the sporocysts of *C. varaniornati*. Both of the aforementioned species are described from hosts that are phylogenetically and geographically distant from the Varanidae.

Kaur and Oberoi (1987) described *Caryospora varani* from *Varanus (Soterosaurus) salvator*, (Laurenti) from India. The description is very poor and, based on given data and pictures, this species does not represent a coccidian parasite and as such should be treated as *nomen nudum*.

The life cycles of the majority of caryosporan coccidia remain unstudied. Two species infecting viperid snakes, *Caryospora bigenetica* and *C. simplex*, have been found to have heteroxenous life-cycle pattern involving rodents as the so-called secondary hosts (Wacha and Christiansen 1982, Upton et al. 1984b, Upton and Barnard 1988). SCID mice were previously shown to be an optimal model for testing the heteroxenous life-cycle pattern of *C. simplex* from viperid snakes (Modrý et al. 1997, Vítovec et al. 1997, Koudela et al. 2000). Failure to infect these model hosts with *C. varaniornati* suggests the possibility that a heteroxenous pattern of the life cycle involving rodent intermediate/secondary hosts is unlikely.

Traditionally, monitor lizards of tropical Africa are divided into two species: the steppe monitor, *Varanus exanthematicus* (Bosc) and the Nile monitor, *Varanus niloticus* (L.), belonging to two different subgenera (Mertens 1942). Recently, it has been shown that both are closely related and belong to single subgenus *Polydaedalus* and that both "species" are actually complexes of distinct forms (Böhme 1997). Members of the *Varanus niloticus* species complex inhabit nearly the entire African continent except for the desert areas. Böhme and Ziegler (1997) mentioned two distinct

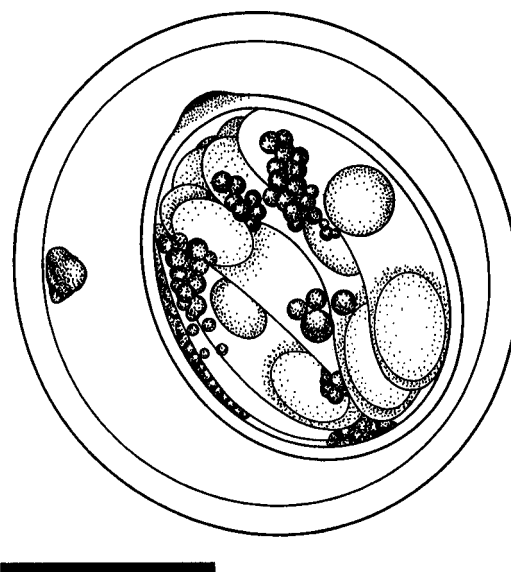


Fig. 4. Composite line drawing of sporulated oocyst of *Caryospora varaniornati* sp. n. Scale bar = 5 μ m.

species differing in morphological, ecological and distributional characters – *Varanus niloticus* and *V. ornatus*. In our experiment, we failed to transmit *C. varaniornati* from naturally infected *Varanus ornatus* to *V. niloticus*; despite the immunosuppression of one experimental animal. The data about host specificity of reptilian coccidia of the family Eimeriidae are scarce; Modrý and Koudela (1995) described in a case of *Isoospora jaracimrmani* the host specificity on the level of single host species. Whereas it is attractive to speculate about the potential strict host specificity of *C. varaniornati*, negative results of the experimental transmission study may also be explained if the oocysts were non-infectious. Experimental infection of *V. ornatus* was impossible due to the unavailability of experimental animals. A life cycle for *C. varaniornati* with an intermediate/secondary host other than murid rodents is possible, but it is premature to postulate a heteroxenous cycle based on the limited data in this study. The life cycle of *Caryospora varaniornati* therefore remains unknown and needs further research.

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