**Sarcocystis stenodactylicolubris** n. sp.,
A NEW SARCOSPORIDIAN COCCIDUM
WITH A SNAKE-GECKO HETEROXENOUS LIFE CYCLE

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**INTRODUCTION**

Snakes are often reported as definitive hosts of the coccidian genus *Sarcocystis* (e.g. Matuschka, 1985; McAllister et al., 1993, 1995, 1996; Paperna & Finkelman, 1998; Upton et al., 1992). Seventeen *Sarcocystis* species are described with a full life cycle (Matuschka, 1987a; Odening, 1998; Šlapeta et al., 1999; Volf et al., 1999). Although some of these species use lizards of saurian families Lacertidae and Scincidae as intermediate hosts, no *Sarcocystis* cycling between snakes and geckoes (Gekkonidae) was described to date. However, a few records of sarcocysts from tissues of gekkonid lizards were reported (Bertram, 1892; Chatton & Avel, 1923; Dupouy & Kechemir, 1973; Paperna & Finkelman, 1998; Weber, 1909, 1910). Presented paper provides the results of series of transmissional experiments with an isolate of *Sarcocystis* originating from a colubrid snake *Coluber najadum*. Furthermore, the endogenous developmental stages of this species are described within its intermediate (gecko) and final (colubrid snake) hosts after an experimental infection. Described *Sarcocystis* differs significantly from all hitherto described members of the genus and is therefore considered to be a new species.

**MATERIALS AND METHODS**

**Infectious material**

An adult, melanistic male of Dahl’s whip snake, *Coluber najadum* (Eichwald, 1831) was collected in black lava desert near Ar Rashiedeh in SW Syria during the herpetological and parasitological field research in April 1994. Snake was trans-
ported to the Department of Parasitology of University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic. The snake was kept in an isolated terrarium and force-fed with sucking laboratory mice. Fresh faecal samples were collected from terrarium and examined repeatedly using modified Sheater’s sugar solution (s.g. 1.30) flotation technique. The snake was euthanized due to bad health status three weeks after importation and tissue samples were fixed for further examination. Sporocysts from the faeces and intestinal content were concentrated by flotation, examined morphologically and photographed using Nomarski interference contrast optics (NIC). Measurements were made using a calibrated ocular micrometer and are reported in micrometers, as means, followed by the ranges in parentheses. Sporocysts were stored at a temperature of 4-5°C in 2.5 % (w/v) aqueous potassium dichromate solution. Prior the inoculation sporocysts were washed in tap water and counted using haemocytometer.

INFECTION
AND MAINTENANCE OF EXPERIMENTAL ANIMALS

Following laboratory bred lizards, which can naturally occur in food spectrum of *Coluber najadum* in Syria, were used for intermediate host studies (numbers of infected and control animals used in experiments are noted in parentheses): Lacertidae: *Acanthodactylus grandis* (2, 1); Mesalina brevirostris (2, 1); Scincidae: *Chalcides ocellatus* (3, 2), *Mabuya vittata* (2, 1); Gekkonidae: *Pyodactylus guttatus* (2, 1), *Stenodactylus grandiceps* (1, 1). All lizards were caged separately in plastic terraria and fed on laboratory reared crickets with mineral and vitamin supplementation. Each experimental lizard was inoculated orally with 2 x 10³ sporocysts by a stomach tube. Additionally, five BALB/c mice and five laboratory reared common voles (*Microtus arvalis*) were infected orally with 10⁵ sporocysts and kept as described previously (Slápet et al., 1999). Experimentally inoculated and control lizards were euthanized by ether and dissected 78 days after the infection (DPI) and experimentally inoculated rodents were examined on 150 and 180 DPI, respectively.

In the definitive host study one adult, laboratory kept, coccidia free colubrid snake *Coluber rogeri* was used. Snake was fed solely on laboratory mice. Faeces were monitored using flotation technique with negative result for 12 months prior the experimental infection. One living, captive born male of *Pyodactylus guttatus*, inoculated orally with 2 x 10⁵ sporocysts 80 days before, was fed to this snake. Terminal half of the tail of this gecko was biopsied two days before the feeding experiment for presence of sarcocysts in muscles. Faecal samples of inoculated snake were examined using flotation method for the presence of coccidia in intervals of 5-15 days, depending upon the irregular feeding habit of the snake.

HISTOLOGY

At necropsies, following tissue samples were collected and fixed in 10 % buffered formalin: naturally infected *Coluber najadum* – seven equidistantly spaced sections of the intestine; lizards – stomach, small intestine, large intestine, cloaca, heart, lung, liver, kidney, abdominal wall, tongue, biceps femoris muscle and tail; rodents – oesophagus, stomach, duodenum, middle jejunum, ileum, caecum, colon, rectum, heart, lungs, liver, spleen, kidney, urine bladder, tongue, diaphragm, heart, muscles of the abdominal wall, brachial muscles and left biceps femoris muscle. Tissue samples were consequently processed for the histology using standard methods. Paraffin sections were stained with haematoxylin and eosin (H & E) and Giemsa stains.

ELECTRON MICROSCOPY

Muscles of *Stenodactylus grandiceps* containing sarcocysts were fixed in 2.5 % glutaraldehyde in cacodylate buffer (0.1 M, pH 7.4) at 4°C and postfixed in 1 % osmium tetroxide in the same buffer. Specimens were washed three times in the same buffer, dehydrated in graded alcohols and embedded in Durcupan. Ultrathin sections were stained with uranyl acetate and lead citrate and then examined with a JEOL 1010 transmission electron microscope. Specimens for scanning electron microscopy (SEM) were fixed in 4 % buffered paraformaldehyde at 4°C. Small portions of tissue were rinsed several times in distilled water, dehydrated in alcohol and acetone series and desiccated by critical point drying in carbon dioxide. The samples were then gold-coated in spraying device and examined with a JEOL JMS 6300 scanning electron microscope.

RESULT

STAGES IN FOUND THE DEFINITIVE HOST

Examination of the intestinal content of naturally infected *Coluber najadum* revealed numerous liberated sporocysts of *Sarcocystis* sp. (Fig. 1). Oocysts were only rarely seen. Sporocysts are tetrazoic, ellipsoidal, 9.7 (9-10) x 7.6 (7-8) μm, shape index 1.3. Stieda and substieda bodies are absent. Sporocyst residuum is present, composed of numerous small granules of the equal size. Sporocyst wall is single-layered, smooth and colourless; sporozoites are long oval. Numerous sporocysts, structurally and morphologically identical with the original isolate, were first found on 39 DPI in faeces of experimentally infected *Coluber*
r. Sarcocysts found in S. grandiceps 78 DPI were elongate, reached a length of 175-200 µm and a width of 35-50 µm. When studied with the light microscope, the primary cyst wall of sarcocysts was about 1-2 µm thick, with barely visible villar protrusions (Fig. 2). Secondary cyst wall was not observed. Elongated cystozoites, 5-6 x 1.5 µm with centrally located nucleus were observed inside cysts, together with few scattered metrocytes.

Ultrastructurally, the sarcocyst wall belongs to the type II, according Dubey et al. (1989). There were characteristic spine-like villar protrusions arising from the cyst wall. Ground substance was ca. 200-300 nm thick with numerous septa stretched dividing the cyst into compartments containing cystozoites (Fig. 5). Protrusions are up to 800 nm long, 200-250 nm in diameter at their base, tapering to thinner apex, ca 100 µm in diameter. The wall of protrusions appeared slightly undulated in longitudinal sections. Protrusions are typically lobular or irregular in the cross-sections, mainly at the basis (Fig. 6). Endodyogony (Fig. 4) was the only reproduction mode observed within examined sarcocysts. Sarcocysts observed in experimentally infected Ptyodactylus guttatus 78 DPI were morphologically identical to that reported above.

**TAXONOMIC SUMMARY**

Sarcocystis stenodactylicolubris n. sp.

**Exogenous stages:** Oocysts only rarely seen, fully sporulated free sporocysts represent a majority of stages found in the faeces. Sporocysts tetrazoic, ellipsoidal, 9.7 (9-10) x 7.6 (7-8) µm. Sporocyst residuum present, composed of numerous small granules of the equal size. Sporocyst wall single-layered, smooth and colourless, with distinct sutures observed by SEM; sporozoites elongately oval.

**Sarcocysts:** Sarcocysts are elongated, 78 DPI are 175-200 µm long and 35-50 µm wide. Sarcocyst wall type II, according Dubey et al. (1989). Primary sarcocyst wall ca. 2 µm thick, with numerous spine-like villar protrusions, lobular or irregular in the cross-section.

**Type host:** Dahl's whip snake, Coluber najadum (Eichwald, 1831) (Serpentes: Colubridae).

**Other hosts:** Coluber r. rogersi (Anderson, 1893) (final, experimentally infected); Stenodactylyus grandiceps Haas, 1952; P. guttatus Heyden, 1827 (intermediate, all infected experimentally).

**Type locality:** Ar Rashiedeh, SW Syria (32° 40'N, 37° 3'E)

**Type specimens:** Photosyntypes and histological slides are deposited in Parasitological Institute of Academy of Sciences of the Czech Republic in České Budějovice, coll. No. R 181/94. Symbiotypes: Symbiotype of the type host (alcohol preserved) is deposited in the Herpetological collec-
Figs 1-6. – Basic morphological features of Sarcocystis stenodactylicolubris sp. n. Fig. 1. Sporocysts isolated from faeces of C. najadum, scale bar = 5 µm. Fig. 2. Histological section of the tail musculature of S. grandiceps with sarcocyst, note barely visible villar protrusions (arrowheads). Fig. 3. Ultrastructure of sarcocyst of Sarcocystis stenodactylicolubris showing several cystozoites, septa and protrusions. Fig. 4. Cystozoites dividing by the endodyogony. Fig. 5. Spine-like villar protrusions of the primary cyst wall, scale bar = 200 nm. Fig. 6. Cross-section through the zone of protrusions showing their lobular character.
Figs 7-12. - Developmental stages of *Sarcocystis stenodactylicolubris* sp. n. in *Coluber najadum*. Fig. 7. Histological section of intestinal mucosa with oocysts/sporocysts (arrows), scale bar = 10 µm. Fig. 8. SEM picture of mucosal surface showing erosions with liberating sporocysts (arrow). Fig. 9. SEM picture of cross section through intestinal mucosa with numerous hollows indicating previous location of developmental stages (arrow). Fig. 10. Detail view of the same region. Fig. 11. Sporocysts liberating in clusters from erosions in the mucosal surface. Fig. 12. Oocyst (arrow) and sporocysts liberated from the intestinal mucosa. Note rib-like prominating sutures of plates of sporocyst wall (arrowheads).
tion of the National Museum Prague, coll. No. NMP6W-34896.

Etymology: The specific name *stenodactylicolubris* reflects the generic name of the intermediate (*Steno-

**DISCUSSION**

coccidia of the genus *Sarcocystis* are common parasites among snakes of family Colubridae, which serve as definitive hosts (Matuschka, 1985, 1987a, McAllister et al., 1993, 1995; Paperna & Finkelman, 1998; Roudabush, 1937; Volf et al., 1999). In contrast to common findings of these parasites in colubrids, only six species were described with their life cycle up to date (Bledsoe, 1980; Matuschka, 1981, 1986, 1987b; Sakran, 1993; Volf et al., 1999). Various lizards are intermediate hosts of four of these species, which are therefore compared with *Sarcocystis steno-

*Sarcocystis gongyi* Trinci 1911 is described from colu-

*Sarcocystis balchidicolubris* Matuschka 1987 described from the Mediterranean region uses also colubrid snake, namely *Coluber ravergieri* as definitive hosts and skink *Chalcides ocellatus* as intermediate hosts. *S. ste-

Sporocysts wall composed of four collapsible plates joined in rib-like prominating sutures was repeatedly reported in members of genera *Isospora*, *Toxoplasma* and *Sarcocystis* (Box et al., 1980; Speer et al., 1973, 1976). Our results showing sporocysts surface of *S. ste-

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thick sarcocyst wall and belong evidently to a species different from *S. platydactylicolubris*. The final host of *S. platydactyli* remains unknown and needs further research. Recently, Paperna & Finkelman (1998) reported presence of sarcocysts in tissues of *Pyodactylyus basselquistii* from Israel. Ultrastructurally, sarco-

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*Sarcocystis lacertae* Babudieri, 1932 cycles between colubrid snake *Coronella austriaca* and lizards *Podarcis muralis*. Also this species differs significantly in its ultrastructure, having spine-like, typically arching protrusions with spongiform appearance of the ground substance (Volf et al., 1999).

A few records of sarcocysts from tissues of gekkonid lizards are reported to date. Bertram (1892) had described *Sarcocystis platydactyli* from Mediterranean gecko *Tarentola mauritanica*. Later on, several authors reported tissue cysts in this common Mediterranean gecko (Weber, 1909, 1910; Chatton & Avel, 1923; Dupouy & Kechemir, 1973). However, tissue cysts reported by all of these authors are typical in having
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