

## Discovery of the life cycle of *Sarcocystis lacertae* Babudieri, 1932 (Apicomplexa: Sarcocystidae), with a species redescription

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**Abstract.** Oocysts/sporocysts of *Sarcocystis* sp. were found in the intestinal contents of the smooth snake, *Coronella austriaca* Laurenti. Common voles *Microtus arvalis* (Pallas), bank voles *Clethrionomys glareolus* (Schreber), green lizards *Lacerta viridis* (Laurenti), and common wall lizards *Podarcis muralis* (Laurenti) were experimentally inoculated as potential intermediate hosts. Only common wall lizards were found to be susceptible intermediate hosts. Transparent, macroscopically hardly visible sarcocysts found in tail striated muscles of lizards were 480 (390-640) × 210 (190-230) µm in size 72 days post-infection. Using the light microscopy, the sarcocyst wall was about 1 µm thick with an apparent layer of villi approx. 2 µm thick. Ultrastructurally, the primary cyst wall was characterised by spine-like villar protrusions up to 2.5 µm in length and 0.5 µm in diameter. Based on sarcocyst morphology and experimental data, the discovered *Sarcocystis* species is suggested to be conspecific with *Sarcocystis lacertae* Babudieri, 1932. A redescription of *Sarcocystis lacertae* is presented in this study.

Description of “*Coccidium*” sp., excreted by the smooth snake, *Coronella austriaca* Laurenti, by Grassi (1881), represents the first report on infection of a reptilian host by coccidia of the genus *Sarcocystis*. Since 1892, reptiles have also been reported as intermediate hosts, harbouring *Sarcocystis* cysts in their muscles (Bertram 1892). A few species use reptiles as both definitive and intermediate hosts, and three have been described to day that possess a snake-lizard life cycle: *Sarcocystis gongyli* Trinci, 1911, *Sarcocystis chalcidicolubris* Matuschka, 1987 and *Sarcocystis podarcicolubris* Matuschka, 1981.

This paper reports on discovery of *Sarcocystis lacertae* Babudieri, 1932 in both the intermediate and definitive host, on recognition of its full life cycle in snakes and lizards and, finally, provides a redescription of this originally poorly defined species.

### MATERIALS AND METHODS

**Infectious material.** An adult, female smooth snake *Coronella austriaca* was found killed on the road at Čabrad (ca. 48°20'N; 19°10'E), Slovak Republic in July 1995. Contents of the posterior portion of the intestine was removed, placed into 2.5% (w/v) aqueous potassium dichromate and submitted for parasitological examination to Department of Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic. Intestinal contents were routinely screened for parasites using flotation in Sheather's sugar solution (s. g. 1.30). Isolated coccidian oocysts/sporocysts were examined and photographed using Nomarski interference contrast microscopy (NIC). Thirty sporocysts

were measured using bright-field microscopy (×100 objective) with a calibrated ocular micrometer. The sporocysts were washed three times in tap water by centrifugation and counted using haemocytometer before they were orally administered to each potential intermediate host.

**Maintenance of experimental animals and experimental transmissions.** To determine the intermediate host, the following experimental animals from the potential food spectrum of *Coronella austriaca* were used for experimental infections (numbers and origin of the animals are listed in parentheses): bank voles *Clethrionomys glareolus* (Schreber) (3, captive born), common voles *Microtus arvalis* (Pallas) (3, captive born), green lizards *Lacerta viridis* (Laurenti) (6, captive born juveniles obtained from private herpeto-keeper) and common wall lizards *Podarcis muralis* (Laurenti) (4, subadult, captive born, obtained from private herpeto-keeper). Rodents were housed in standard plastic cages with wooden shavings as bedding and were kept on standard rodent diet and water *ad libitum*. The lizards were kept in glass or plastic terraria fitted with heating lamps and were fed on laboratory reared crickets powdered with vitamins and mineral supplements.

All experimental animals were orally (p. o.) inoculated with 10<sup>3</sup>-10<sup>4</sup> sporocysts using a stomach tube. All animals were monitored daily for clinical signs of disease or death due to sarcosporidian infection. Individuals of all species were euthanized by overdosing with barbiturate (Thiopental®, Spofa, Czech Republic) and necropsied on the following days post infection (DPI, in parentheses): *Clethrionomys glareolus* (30, 60), *Microtus arvalis* (111, 139), *Lacerta viridis* (10, 21, 21, 49, 49) and *Podarcis muralis* (72, 72, 72). One animal of each species served as an uninfected control.

For the experimental back-transmissions of *Sarcocystis lacertae*, a captive Rogers's whip snake, *Coluber rogersi* (Anderson) and a *Coronella austriaca* Laurenti (both Colubridae) were used. Lizards *Podarcis muralis*, inoculated with  $3 \times 10^3$  sporocysts 72 days in advance, were fed to both snakes. Distal portions of tails of both lizards were found to contain numerous sarcocysts during native and subsequent histological examination. Snakes were kept in plastic terraria with a heating lamp and fed weekly on suckling laboratory mice. Faecal samples were collected from the bottom of terraria and examined using the flotation technique as described above. Faecal samples of *Coluber rogersi* were collected at 5, 21, 70 and 86 DPI, and those of *Coronella austriaca* were collected at DPI 2, 33, 39, 61, 78, 99 and 114. Finally, *Coronella austriaca* was euthanized at 154 DPI and necropsied in the same way as experimental lizards.

**Light microscopy and transmission electron microscopy.** For histological examination, the following tissues were collected from inoculated rodents and fixed in 10% buffered formalin: oesophagus, stomach, duodenum, jejunum, ileum, caecum, rectum, lung, liver, kidney, spleen, mediastinal lymphatic nodes, tongue, heart, diaphragm, muscles of the abdominal wall, brachial muscles (m. triceps brachii), thigh muscles (m. quadriceps femoris) and masseters. Tissue samples from experimental lizards were collected as follows: oesophagus, stomach, small intestine, large intestine, heart, lung, liver and tail. The small intestine of experimentally infected smooth snake was divided into four parts, fixed immediately and prepared for histology. Fixed tissues were processed by standard histological methods. Paraffin sections were stained with haematoxylin and eosin (HE) and examined by light microscopy.

Additionally, four *Podarcis muralis* were collected at the same locality as *Coronella austriaca* (Čabrad', Slovak Republic) in August 1996. Their tail muscles were obtained by biopsy using the lizard's ability of tail autotomy, and lizards were then released back into the wild. The distal portion of the tails were fixed in 10% buffered formalin and processed for histology. Additional transmission electron microscopy of infected muscles was performed on formalin-fixed tissues retrieved from paraffin blocks. The tail tissues were further fixed in 2.5% glutaraldehyde in cacodylate buffer (0.1M, pH 7.4) at 4°C and processed as described below.

The sarcocysts isolated from experimentally inoculated lizard tail musculature were also homogenised in phosphate buffered saline (PBS, pH 7.2), the centrifuged sediment air dried, fixed with methanol, stained with Giemsa, and cystozoites then measured using a calibrated ocular micrometer.

For transmission electron microscopy, infected tissues were fixed in 2.5% glutaraldehyde in cacodylate buffer at 4°C and post-fixed 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. Thin sections were stained with uranyl acetate and lead citrate and then examined with a JEOL 1010 transmission electron microscope.

## RESULTS

From the spectrum of inoculated potential intermediate hosts, only common wall lizards *Podarcis muralis* were found to be susceptible to infection. Sarcocysts were detected in all *P. muralis* specimens previously inoculated with sporocysts isolated from the intestinal content of smooth snake, *Coronella austriaca*. Consequently, *C. austriaca* fed with tail musculature of previously experimentally infected lizards excreted sporulated oocysts/sporocysts in faeces. No oocysts/sporocysts were found during repeated examinations of the experimentally inoculated Rogers's whip snake, *Coluber rogersi*.

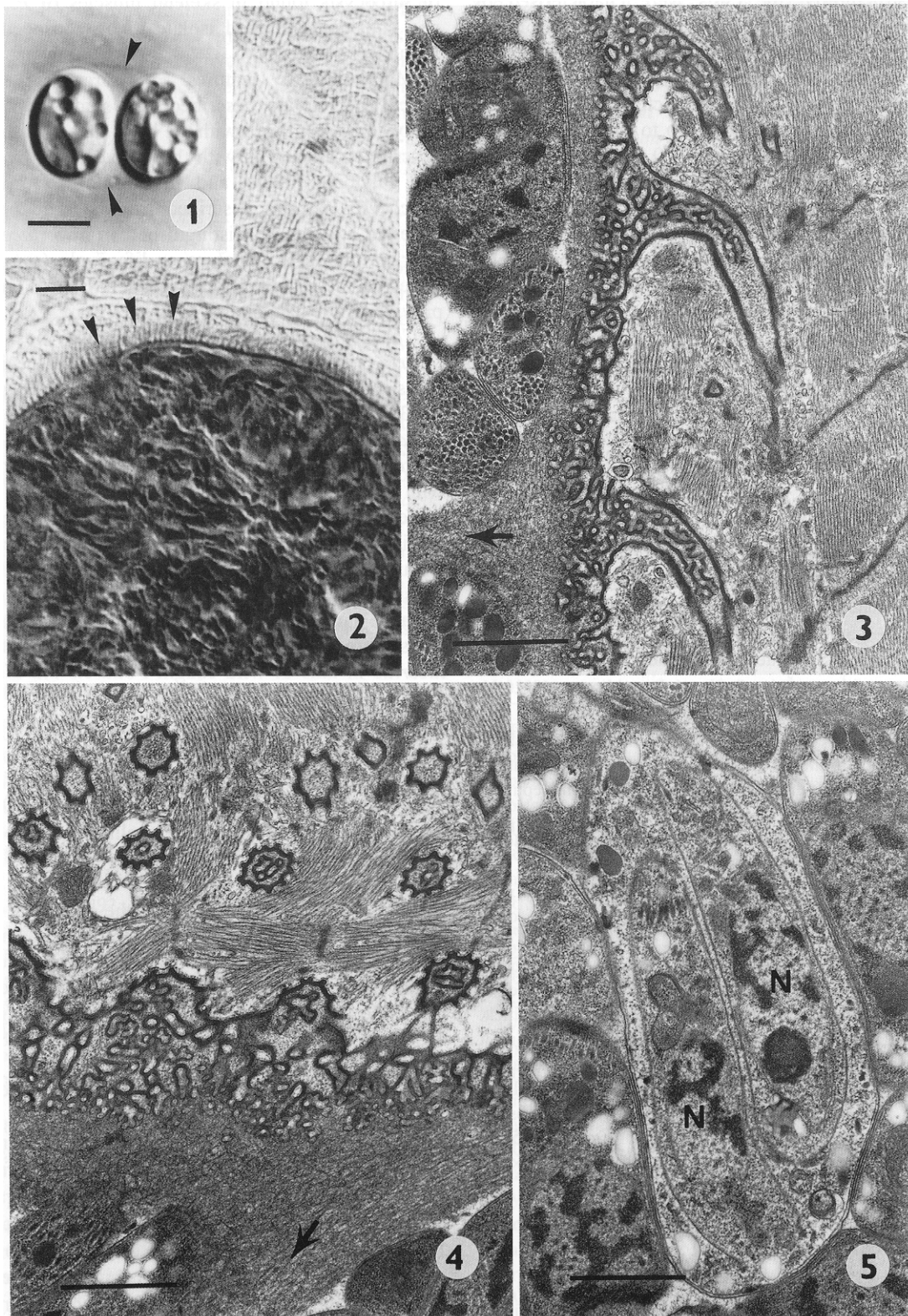
### Stages in definitive hosts

Examination of intestinal content of *Coronella austriaca* from Čabrad' revealed numerous sporulated oocysts/sporocysts of *Sarcocystis* sp. Sporulated oocysts possessed a thin wall, closely surrounding two sporocysts (Fig. 1). Most oocysts were ruptured and liberated sporocysts were observed. Sporocysts were tetrazoic, ellipsoidal,  $9.3 (9.0-10.0) \times 7.5 (7.0-8.0) \mu\text{m}$ , with a shape index (length/width) 1.25 (1.13-1.33) ( $n = 30$ ). Stieda and substieda bodies were absent. The sporocyst residuum was composed of numerous small granules 1.5-2.0  $\mu\text{m}$  in diameter. The sporocyst wall was single-layered, smooth and colourless. Sporozoites were banana-shaped,  $6.4 (6.0-7.0) \times 2.1 (2.0-2.5) \mu\text{m}$  (in situ).

Starting at DPI 33, sporulated oocysts and sporocysts were found in faeces of the experimentally inoculated *Coronella austriaca*. The sporocysts/oocysts were morphologically identical to those isolated from naturally infected *C. austriaca* from the type locality. Histological examination of tissue samples of experimentally inoculated smooth snake revealed numerous gamogonic stages and oocysts/sporocysts of *Sarcocystis* sp. located in lamina propria of the middle part of the small intestine.

### Stages in intermediate hosts

Examination of muscle samples from tails of *Podarcis muralis* experimentally infected 72 days in advance revealed transparent, oval, by naked eye hardly visible sarcocysts measuring  $480 (390-640) \times 210 (190-230) \mu\text{m}$  in fresh muscles. In histological sections, 1-4 sarcocysts per tail cross section were observed. The sarcocyst wall was about 1  $\mu\text{m}$  thick, with a distinct layer of villi about 2  $\mu\text{m}$  thick, giving the wall striated appearance (Fig. 2). Numerous septa stretched from the cyst wall into the cysts. Cystozoites in smear were oval, slightly curved,  $6.2 (6.0-6.5) \times 2.2 (2.0-2.5) \mu\text{m}$  with centrally located nucleus. No inflammatory reaction around the sarcocysts was observed.



**Figs. 1-5.** Morphological features of *Sarcocystis lacertae*. **Fig. 1.** Sporulated oocyst. Note thin oocyst wall (arrowheads). **Fig. 2.** Histological section of apical part of matured sarcocyst in NIC microscopy. Note distinct layer of villar protrusions (arrowheads); HE. **Fig. 3.** Spine-like villar protrusions regularly arising from the cyst wall and then running parallel to the sarcocyst surface; TEM. **Fig. 4.** Cross section of the protrusions with invaginations; TEM. **Fig. 5.** Advanced stage of endodyogeny showing two completely formed daughter cells in the mother parasite cell; TEM. Scale bars: Figs. 1, 2 = 5  $\mu$ m; Figs. 3-5 = 1  $\mu$ m.

Ultrastructurally, the cysts were characterised by spine-like villar protrusions regularly arising from the cyst wall, and then arching 90 degrees and running parallel to the sarcocyst surface (Fig. 3). The villar protrusions, wavy in the cross sections, were 0.5 µm wide at their base and reached up to 2.5 µm in length. The ground substance was 0.5-2.0 µm thick, peripherally pervaded by numerous invaginations, giving it a spongiform appearance. These minute invaginations extended ca. 1 µm into the villar protrusions (Fig. 4). Septa were 0.5-2.0 µm thick. One type of asexual multiplication, the endodyogeny producing two progeny within the parasite cells, was found in sarcocysts (Fig. 5).

Two out of four tails of *Podarcis muralis* originated from the same locality as the naturally infected smooth snake originated from were found to contain sarcocysts, histologically and ultrastructurally identical with those found in tails of experimentally infected lizards.

### Taxonomic summary

***Sarcocystis lacertae*** Babudieri, 1932 Figs. 1-5

Type host (intermediate): common wall lizard, *Podarcis muralis* (Laurenti, 1768) (Sauria: Lacertidae).

Definitive host: smooth snake, *Coronella austriaca* Laurenti, 1768 (Serpentes: Colubridae).

Type locality: Čabrad', Slovak Republic (ca. 48°20'N; 19°10'E).

Neotype material: Phototypes and histological slides are deposited in the parasitological collection of Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, col. Nos. R198/95 and H 4/98.

Diagnosis: Matured sarcocysts oval, 480 (390-640) × 210 (190-230) µm, localised in tail musculature. Cysts wall about 1 µm thick, with a distinct 2 µm thick layer of villi, giving the wall striated appearance. Cystozoites oval, slightly curved, 6.2 (6.0-6.5) × 2.2 (2.0-2.5) µm with centrally located nucleus. Ultrastructurally, the sarcocyst wall with spine-like villar protrusions running parallel to the sarcocysts surface. Ground substance 0.5-2.0 µm thick, peripherally pervaded by numerous invaginations, giving it a spongiform appearance. Oocysts with two tetrazoic sporocysts. Liberated sporocysts ellipsoidal, 9.3 (9.0-10.0) × 7.5 (7.0-8.0); Stieda and substieda bodies absent. Sporocyst residuum present consisting of granules 1.5-2.0 in diameter. Banana-shaped sporozoites 6.4 (6.0-7.0) × 2.1 (2.0-2.5) (in situ).

### DISCUSSION

*Sarcocystis lacertae* was originally described by Babudieri (1932) from the musculature of a common wall lizard *Podarcis muralis* (originally *Lacerta muralis*) in Lombardia, Italy. Only basic morphological data on sarcocysts were given in the original description. More than 30 years later, Sénaud and Puytorac (1964) and Sénaud (1967) studied the structure

of sarcocysts found in skeletal muscles of *P. muralis* and considered studied isolate to be conspecific with Babudieri's material.

Morphological features of sarcocysts of the *Sarcocystis* isolate described and studied within our study correspond well with the basic data of Babudieri's description as well as with those given later by Sénaud and Puytorac (1964) and Sénaud (1967). All other *Sarcocystis* species described from lacertid hosts are different and can be distinguished from *S. lacertae* by their morphologic structure or life cycle (see below). Based on these facts, we retained the original name and provided more data on this species.

*Coronella austriaca*, the definitive host of *S. lacertae*, is mostly saurophagous and the species also feeds occasionally on small mammals (Engelmann 1993). In the studied locality, common wall lizards, *Podarcis muralis*, sand lizards, *Lacerta agilis* and green lizards, *L. viridis* (all Lacertidae) occur syntopically with *C. austriaca*. Although there are no detailed data on feeding preferences of *C. austriaca* in this region, it is highly probable that *P. muralis* is consumed most frequently due to the size and the highest population density. The infectivity of *S. lacertae* for *Lacerta agilis* remains unresolved. Results of experimental transmissions correspond well also with finding of cysts of *S. lacertae* in tails of wild caught *Podarcis muralis* from the type locality.

Babudieri (1932) as well as Sénaud and Puytorac (1964) reported *S. lacertae* from geographical regions where *Coronella austriaca* and *Podarcis muralis* occur sympatrically (Guillaume 1997, Strijbosch 1997) and the natural life cycle in these localities could occur in the same way as in the type locality designed in this study.

Having the information on the full life cycle, *Sarcocystis lacertae* could be compared with other *Sarcocystis* species with the snake-lizard life cycle. Up to day, three species of *Sarcocystis* with snake-lizard life cycle have been described and named: *S. gongyli* Trinci, 1911, *S. podarcolubris* Matuschka, 1981, and *S. chalcidicolubris* Matuschka, 1987 (Trinci 1911, Matuschka 1981, 1987a,b Matuschka and Mehlhorn 1984, Abdel-Ghaffar et al. 1990).

*Sarcocystis gongyli*, described from Mediterranean colubrid snakes and scincid lizards, differs from *S. lacertae* not only in the host range and geographical distribution, but also in the ultrastructure of sarcocysts. The primary sarcocyst wall of this species possesses long, leaf-like protrusions with a remarkable leafstalk (Abdel-Ghaffar et al. 1990) significantly different from the spine-like protrusions typical of *S. lacertae*.

*Sarcocystis chalcidicolubris* Matuschka, 1987 differs from *S. lacertae* not only in the host range, (snakes of the genus *Coluber* and scincid lizards of the genus *Chalcides*), but also in the cyst ultrastructure and size of

cystozoites. Protrusions of the primary cyst wall of *S. chalcidicolubris* are about 2 µm in length, looking dotted because of numerous invaginations (Matuschka 1987b). Protrusions of *S. lacertae* are much thinner and spine-like, with a spongiform ground substance. Additionally, cystozoites of *S. chalcidicolubris* are apparently longer than those of *S. lacertae* (10.0-12.0 µm vs. 6.0-6.5 µm).

*Sarcocystis podarcolubris* is similar to *S. lacertae* in the host range, involving lacertid lizards as intermediate and colubrid snakes as definitive hosts. Numerous lacertid species (11 species, including *Podarcis muralis* and *Lacerta viridis*) are susceptible to infection with *S. podarcolubris*. In contrary, it was impossible to infect *L. viridis* with *S. lacertae* during our experiments. *Sarcocystis podarcolubris* and *S. lacertae* differ also in the definitive host range. Although the spectrum of definitive hosts of *S. podarcolubris* is relatively wide (involving three genera of colubrid snakes), it was not transmissible to *Coronella austriaca* (Matuschka 1985, 1987a). On the other hand, the infection of Rogers's whip snake, *C. rogersi* with *S. lacertae* failed in the present study. Additionally, *S. podarcolubris* and *S. lacertae* could be easily distinguished also on the ultrastructural level. The cysts

of *S. podarcolubris* possess palisade-like protrusions, apparently different from the spine-like protrusions of *S. lacertae*.

Sporocysts of *Sarcocystis* sp. found in faeces of *Coronella austriaca* have already been reported by Grassi as *Coccidium* sp. (Grassi 1882, 1883, Upton 1992). The conspecificity of Grassi's finding with *Sarcocystis lacertae* is questionable since it is hardly possible to use sporocyst/oocyst morphology as a criterion for species determination (Dubey et al. 1989). Similarly, data on sarcosporidian cysts from muscles of *Podarcis muralis* mentioned by Lühe (1900), without any further specification of morphology, site of infection or locality are insufficient to be compared with *Sarcocystis lacertae*. Nevertheless, both aforementioned findings could be tentatively placed into the synonymy of this species.

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## REFERENCES

- ABDEL-GHAFFAR F., BASHTAR A.R., ASHOUR M.B., SAKRAN T. 1990: Life cycle of *Sarcocystis gongyli* Trinci 1911 in the skink *Chalcides ocellatus ocellatus* and the snake *Spalerosophis diadema*. Parasitol. Res. 76: 444-450.
- BABUDIERI B. 1932: I sarcosporidi e le sarcosporidiosi. Arch. Protistenkd. 76: 421-580.
- BERTRAM, A. 1892: Beiträge zur Kenntnis der Sarkosporidien. Zool. Jahrb. Abt. Anat. 5: 581.
- DUBEY J.P., SPEER C.A., FAYER R. 1989: Sarcocystosis of Animals and Man. CRC Press, Boca Raton, 215 pp.
- ENGLMANN W.E. 1993: *Coronella austriaca* (Laurenti, 1768) – Schlingnatter, Glatt- oder Haselnatter. In: W. Böhme (Ed.), Handbuch der Reptilien und Amphibien Europas, Vol. 3/I, Schlangen I. Aula, Wiesbaden. pp. 200-245.
- GUILLAUME C.P. 1997: *Podarcis muralis*. In: J.P. Gasc et al. (Eds.), Atlas of Amphibians and Reptiles in Europe. SEH, Paris, pp. 286-287.
- GRASSI B. 1882: Intorno ad alcuni protisti endoparassitici ed appartenenti alle classi dei Flagellati, Lobosi, Sporozoi e Ciliati. Atti Soc. Ital. Sci. Nat. Mus. Civ. Storia Nat. Milano 24: 135-224.
- GRASSI B. 1883: Sur quelques protistes endoparasites appartenant aux classes des Flagellata, Lobosa, Sporozoa et Ciliata. Arch. Ital. Biol. 3: 23-36.
- LÜHE M. 1900: Ergebnisse der neueren Sporozoenforschung. Zusammenfassende Darstellung mit besonderer Berücksichtigung der Malariaparasiten und ihrer nächsten Verwandten. Zentralbl. Bakteriol. Abt. 1 28: 323-324.
- MATUSCHKA F.-R. 1981: Life cycle of *Sarcocystis* between poikilothermic hosts. Lizards as intermediate hosts for *S. podarcolubris* sp. nov., snakes function as definitive hosts. Z. Naturforsch. 36: 1093-1095.
- MATUSCHKA F.-R. 1985: Experimental investigations on the host range of *Sarcocystis podarcolubris*. Int. J. Parasitol. 15: 77-80.
- MATUSCHKA F.-R. 1987a: Reptiles as an intermediate and/or final hosts of Sarcosporidia. Parasitol. Res. 73: 22-32.
- MATUSCHKA F.-R. 1987b: *Sarcocystis chalcidicolubris* n. sp.: recognition of the life cycle in skink of the genus *Chalcides* and snakes of genus *Coluber*. J. Parasitol. 72: 1014-1018.
- MATUSCHKA F.-R., BANNERT B. 1987: Cannibalism and autotomy as predator-prey relationship for monoxenous Sarcosporidia. Parasitol. Res. 74: 88-93.
- MATUSCHKA F.-R., MEHLHORN H. 1984: Sarcocysts of *Sarcocystis podarcolubris* from experimentally infected Tyrrhenian wall lizard (*Podarcis tiliguerta*), *S. gallotiae* from naturally infected Canarian lizard (*Gallotia galloti*) and *S. dugesii* from Madeiran lizard (*Lacerta dugesii*). Protistologica 20: 133-139.
- SÉNAUD J. 1967: Contribution à l'études des Sarcosporidies et des Toxoplasmes (Toxoplasmea). Protistologica 3: 167-232.
- SÉNAUD J., PUYTORAC P. 1964: Observation de la sarcosporidie du lézard (*Lacerta muralis*). Arch. Zool. Exp. Gen. 104: 182-186.

- STRIJBOSCH H. 1997: *Coronella austriaca*. In: J.P. Gasc et al. (Eds.), Atlas of Amphibians and Reptiles in Europe. SEH, Paris, pp. 344-345.
- TRINCI G. 1911: Nota sopra una *Sarcocystis* parassita di *Gongylus ocellatus* Wagl., con considerazioni critiche sulla morfologia e sulla biologia dei Sarcosporidi. Monit. Zool. Ital. 22: 309-326.
- UPTON S.J., McALLISTER C.T., TRAUTH S.E., BIBB D.K. 1992: Description of two new species of Coccidia (Apicomplexa: Eimerioida) from flat-headed snakes, *Tantilla gracilis* (Serpentes: Colubridae) and reclassification of misnomer species within the genera *Isospora* and *Sarcocystis* from snakes. Trans. Am. Microsc. Soc. 111: 50-60.

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