

Review

# *Sarcocystis* Species (Apicomplexa, Eucoccidiorida) Parasitizing Snakes

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**Abstract:** The genus *Sarcocystis* has approximately 200 species that are able to infect homeothermic and poikilothermic animals. So far, 23 *Sarcocystis* spp. have snakes as definitive hosts (DHs) and mammals and/or reptiles as intermediate hosts (IHs). Some of these species cause disease or even death in their IH. At least two *Sarcocystis* spp. from snakes have relevance in public health. *Sarcocystis nesbitti* causes serious myopathy in humans, and *S. singaporensis* is lethal for rats and was successfully tested in the biological control of these rodents. Molecular identification was not reported for half of *Sarcocystis* spp. shed by snakes. For some snake species, their role as DHs for *Sarcocystis* spp. is totally unknown, including certain snakes which are bred as companion animals. The aim of this review is to discuss the role of snakes as DHs or IHs of *Sarcocystis* spp. and the future directions for the identification of the parasites and the elucidation of their life cycles.

**Keywords:** Sarcocystidae; reptile; sporocyst; bradyzoite; sarcocyst; small mammal; lizard; definitive host; intermediate host



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## 1. Introduction

*Sarcocystis* spp. are cyst-forming parasites of the phylum Apicomplexa, class Sporozoa, subclass Coccidiasina, order Eucoccidiorida and family Sarcocystidae. Parasites of the genus *Sarcocystis* infect a large spectrum of vertebrate homeothermic and poikilothermic animals, including mammals, birds and reptiles [1]. The family Sarcocystidae encompasses the genera *Sarcocystis*, *Toxoplasma*, *Besnoitia*, *Hammondia*, *Neospora* and *Cystoisospora* [2]; the genus *Frenkelia* used to belong to the family Sarcocystidae, but phylogenetic studies led to its placement in the genus *Sarcocystis* [3–5]. The family Sarcocystidae can be divided in two categories: parasites with an obligate two-host life-cycle, including *Hammondia* spp. and *Sarcocystis* spp. [6,7], and those with a facultative two-host life-cycle, such as *Toxoplasma gondii* and *Neospora caninum* [8]. A unique characteristic of *Sarcocystis* spp. is the sporulation of oocysts in the intestinal tract of their DHs; as their oocysts possess a fragile and delicate wall, fully sporulated sporocysts are released from oocysts in the intestinal lumen of the DHs and represent the predominant parasitic stage shed in their feces. Oocysts of other Sarcocystidae genera are shed in the nonsporulated form in the feces of the DHs [8].

The DHs of *Sarcocystis* spp. are carnivores or omnivores who are capable of preying on and ingesting encysted bradyzoites in tissues of the preyed-on animals. Bradyzoites ingested by a DH undergo other parasitic stages that culminate with the formation of infective oocysts and sporocysts in their intestinal tracts. Approximately one fourth of the over 200 described species of *Sarcocystis* sp. have domestic or wild canids and felids as DHs; until 2016, complete life cycles were known for 26 *Sarcocystis* spp. [1]. In wildlife, an increasing number of predator reptiles have been identified as DHs of this parasite, including snakes. The aim of this review is to discuss the role of snakes as DHs and IHs of *Sarcocystis* spp. and their potential impact on animal and public health.

## 2. Materials and Methods

The literature search for any paper related to *Sarcocystis* spp. in snakes was carried out using the following databases: Scopus "<https://www.scopus.com/>" (accessed on 2 May 2023), Pubmed "<https://pubmed.ncbi.nlm.nih.gov/>" (accessed on 2 May 2023) and Google Scholar "<https://scholar.google.com/>" (accessed on 2 May 2023). A broad number of keywords was used and included *Sarcocystis*, Sarcocystidae, sarcocyst, sarcosporidiosis, sarcocystosis, and cyst-forming, which were combined with snake and reptile. Articles in English and in other languages were retrieved from these databases, as well as textbooks that were related to the topic of the current review.

## 3. Snakes as Definitive Hosts of *Sarcocystis* spp.

In the early 1970s, the role of snakes as DHs of *Sarcocystis* sp. was demonstrated in Australia [9]; sporocysts of the parasite were shown in the feces of five Australian carpet pythons (*Morelia spilotes variegata*). Intestinal scrapings of the snakes had fully sporulated oocysts, which contained delicate walls and sporulated sporocysts. The bush rat (*Rattus fuscipes*) was suspected to be the IH of the parasite, as a high percentage of these rats had previously been confirmed to have high rates of sarcocysts in their skeletal and subcutaneous musculature [9]. Therefore, it has been hypothesized that reptiles could also participate in the life cycle of certain species of *Sarcocystis*. These findings were connected to the discovery of cysts that seemed to be *Sarcocystis* sp. in bush rats (*Rattus fuscipes*) from the same region. Both rat and snake had formerly been associated in the food chain, as they were shown to participate in a nematode life cycle as IHs and DHs of the parasite, respectively. The initial hypothesis was validated through a successful laboratory experiment using Norway rat (*Rattus norvegicus*) [9].

Also in the 1970s, *Sarcocystis singaporensis* was identified in snakes, whose original species name was *S. orientalis* [10,11]. *Sarcocystis singaporensis*, which has reticulated python (*Python reticulatus*) as its main DH, was further re-described, and two additional species were identified: *S. zamani* and *S. villivillosoi* [12]. Up to now, *S. singaporensis* has been one of the most studied species in snakes due to its endemic situation in Southeast Asia, and for causing death in certain species of rats [13–15]. Through experimental infections, Timor python (*Python timorensis*), black headed python (*Aspidites melanocephalus*) and African rook python (*Python sebae*) were identified as additional DHs of *S. villivillosoi* and *S. singaporensis* [16].

*Sarcocystis murinotechis* was classified in tiger snakes (*Notechis ater*) from Australia and was shown to have different species of murids to IHs [17]; it was differentiated from *S. singaporensis* and Rzepchzyk's *Sarcocystis* sp., which also have rats as the IH. Since the DH of *S. singaporensis* did not occur in Australia, experimental infections were performed to identify the hosts of *S. murinotechis*. The DH of *S. murinotechis*, the tiger snake *Notechis ater*, is the sole representative of the Family Elapidae of *Sarcocystis* spp. infecting snakes, and was proved to be a different species from *S. singaporensis* [17].

One of the most important species that cycles in snakes is *Sarcocystis nesbitti*, which is zoonotic and apparently endemic in Southeast Asia; it causes muscular sarcocystosis in humans [18–20]. Based on phylogenetic evidence suggesting that snakes could be the DH of this parasite [21], studies were conducted to confirm this hypothesis. Molecular evidence of *S. nesbitti* in snakes' stools reinforced this assumption. The reticulated python (*Python reticulatus*) and the monocled cobra (*Naja kaouthia*) were considered putative DHs for this species, but no sporocysts were observed in their feces [22,23]. Potential sporocysts of *S. nesbitti* were detected in scrub python (*Simalia amethystina*) in Australia [13]. This was an unexpected finding due to the geographical barrier and the lack of the stated IHs of this species in the Australian territory. Thus far, the DH of *S. nesbitti* in Southeast Asia remains unknown.

Viperid snakes are only hosts for *Sarcocystis* spp. in the snake–rodent life cycle. Most of these species were detected in the African continent. The Viperidae family is divided into two subfamilies: Viperinae and Crotalinae. Specimens of the former are found in Africa, Asia and Europe, and the latter in Asia and in the Americas [24]. *Sarcocystis crotali* was identified in Mojave rattlesnake (*Crotalus scutulatus scutulatus*) in the United States [25]. Little is known about *Sarcocystis* spp. parasitizing members of the subfamily Crotalinae, but some studies were reported in the United States and Brazil [26–29].

Snakes of the genus *Bittis* serve as DHs for *Sarcocystis dirumpens* and *Sarcocystis hoarensis* [30,31]. *Sarcocystis hoarensis* had previously been believed to be *Besnoitia* sp., due to the localization of the cysts and to its macroscopic aspect in the skin [32]; ultrastructural studies of its sarcocyst showed typical characteristics of *Sarcocystis* sp. and it was reclassified in this genus [30]. Out of the few biological experiments performed with this family group, *S. atheridis* and *S. gerbilliechis* exhibited certain host specificity [33,34]. The latter showed a narrow specificity with the species level, and *S. atheridis* to the genus. Nevertheless, regarding the employed experimental host range, it would be necessary to enlarge the spectrum of tested animals to confirm this observation. *Sarcocystis muriviperae* was identified in two animals of distinct families, and seems to be the only named species less specific to the DH [35–37], as infection by *Sarcocystis nesbitti* in *Naja kaouthia* was not truly confirmed [22,23].

Colubrid snakes act as DHs of a great number of *Sarcocystis* species. Up to now, for all *Sarcocystis* spp. that share a snake–reptile life cycle, the DH has been found as a snake of the family Colubridae. Seven species of this family also cycle between mammals [1,38,39]. This fact may be related to the wide distribution of Colubridae, since it is recognized as the most voluminous taxon in the order Squamata, with a great number of genera and species [40,41]. Most of the *Sarcocystis* spp. of colubrids were detected in Africa, Europe and Asia. A few reports were from the Americas. In the snake–rodent cycle, *S. idahoensis* and *S. pantherophisi* are reported. *Sarcocystis idahoensis* has the Great Basin gopher snake (*Pituophis melanoleucus deserticola*) as DH [42,43], whereas *S. pantherophisi* has the eastern rat snake (*Pantherophis alleghaniensis*) as DH [38]. Their major importance relies in the induction of clinical signs in their IH (Section 3).

*Sarcocystis* spp. life cycles in colubrids, in the snake–rodent or in the snake–reptile cycles, was demonstrated to be more specific to the DH in the level of genus or even species than its IH [44,45]. Some snake species are hosts for more than one species of *Sarcocystis*, a fact that had already been observed in other *Sarcocystis* spp. in different hosts [12,16,44].

Based on current knowledge, *Sarcocystis zuoi* was the only species with strict host specificity; the king rat snake (*Elaphe carinata*) is its only known DH [45]. This might be related to the lack of experimental infections in other species such as *S. pantherophisi* [38] and *S. attenuati* [39], for example. In an experiment performed by Hu et al. [45], towards the identification of the DH for *S. zuoi*, the beauty snake (*E. taeniura*) was ruled out of its host range. Later, this snake species was identified as the natural DH of *S. attenuati* in Asia. Although they had been classified as different species with distinct sarcocyst wall morphologies, they had similar 18S rDNA and ITS1 sequences [39]. *Sarcocystis attenuati* and *S. clethrionomyelaphis* share the same DH, *Elaphe taeniura*, and they were isolated from the same geographical region in China. However, they differ in morphology, and phylogenetically they are placed in distinct subgroups based on 18S rDNA.

Species such as *S. clethrionomyelaphis* and *S. muriviperae* are the exceptions to the DH specificity, as they use three and two different genera as hosts, respectively; *S. muriviperae* also use animals of distinct families of snakes as DHs. Nevertheless, when *S. clethrionomyelaphis* was first identified, all natural and experimental species belonged to the genus *Elaphe* [46], but its taxonomy later changed. Thus, nowadays, three genera are reported as DHs. Moreover, evidence indicates that this species may be widely spread, regarding the large range of DHs native to distinct regions, associated with the fact that it was detected in two continents [46,47].

Results of experimental studies with *S. muriviperae* demonstrated an interesting fact in terms of its IHs. Sporocysts obtained from naturally infected Palestine viper (*Vipera palaestinae*) and black whipsnake (*Coluber jugularis*) demonstrated differences in the applied doses to induce the same clinical signs in the early stages of merogony [35]. Although they were assumed to be the same species, morphologically and biologically, a cross-infection experiment using both isolated species was not performed to elucidate whether they are in fact the same species. Molecular analysis would help to clarify the discrepant results following infection in the DH.

The genus *Coluber* sp. was the first snake taxon related to the snake–reptile cycle [48] and it is known to harbor three species of *Sarcocystis*, besides *S. muriviperae*: *S. podarcicolubris* [48], *S. chalcidicolubris* [49] and *Sarcocystis stenodactylolubris* [50]. Although they have the DH in common, they differ in morphological characteristics of the sarcocyst wall and in the parasite’s biology. *Sarcocystis chalcidicolubris* [49] and *Sarcocystis stenodactylolubris* [50] were observed in lizards of different genera and families, respectively; they differ from those *Sarcocystis* spp. previously reported [48,51]. The distinction between these species and *S. podarcicolubris* was based on sarcocyst morphology and the parasite’s biology. Biologically, the IHs of *S. chalcidicolubris* did not acquire infection after ingesting sporocysts from *S. podarcicolubris*; *S. stenodactylolubris* was only infective to a genus of the gekkonidae family, indicating that they are different species.

*Sarcocystis lacertae* has an IH in common with *S. podarcicolubris*. However, experimental infections involving the latter species evidenced that specimens of the genus *Malpolon*, *Elaphe* and *Coronella* were not infected under experimental infections with *S. podarcicolubris* [51]. Posteriorly, the smooth snake (*Coronella austriaca*) was identified as the natural DH for *Sarcocystis lacertae* [52]. *S. lacertae* DNA was detected in the feces of a black-headed cat snake (*Boiga nigriceps*) from Malaysia [53], but sporocysts were not seen in the stools. Regarding geographic barrier, as the genus *Podarcis* does not occur in Southeast Asia [54], this finding may be related to another species closely related to *S. lacertae* or the IH might be other Lacertidae species in the island.

Through experimental assessment, the diadem snake (*Spalerosophis diadema*) was found to be the DH for *Sarcocystis gongyli*; besides sharing the same IH genus of *S. chalcidicolubris*, they differ in DHs and sarcocysts’ morphology [55]. The diadem snake was also identified as the DH of *Sarcocystis acanthocolubri* through transmission experiments [56], and as in the experiment performed for *S. gongyli* [55], the other two tested snakes were not infected.

All the above-mentioned species that use other reptiles as IHs were found in Europe, Africa and Asia. Nowadays, there are a few species of *Sarcocystis* classified in the Americas [38,42,43], and just one reported to have a snake–lizard cycle, namely *Sarcocystis ameivamastigodryasi* [57]. This species, described in Brazil, was the first reported of reptiles as IHs of *Sarcocystis* sp. from snakes in the Americas. The Boddaert’s tropical racer (*Mastigodryas boddaerti*) and the swamp racer (*M. bifossatus*) act as DHs. In this experiment, one specimen of the common boa (*Boa constrictor*) was tested; however, it was not infected [57]. *Sarcocystis* spp. that use snakes as DHs are summarized in Tables 1 and 2.

**Table 1.** Snakes as definitive hosts of *Sarcocystis* spp. and mammals as their intermediate hosts.

<i>Sarcocystis</i> Species	Snake Species (Family)	Intermediate Host	Sarcocyst Description/Animal Tissue	Place of Sampling	Molecular Marker	Reference
<i>S. attenuati</i>	<i>Elaphe taeniura</i> (Colubridae)	<i>Crocidura attenuata</i> <sup>a</sup>	740–1355 × 117–250 µm, thick wall (3.3–4.5 µm), type 9h, protrusions 3.3–4.5 µm, GS 1.2–1.6 µm, bradyzoite 8.2–10.4 × 2.0–3.0 µm/esophagus, diaphragm, tongue, skeletal muscles (thigh, loin, rump, and ribs) and heart	Yunnan Province, China <sup>a</sup>	18S rDNA, Cox1, Cox3, ITS1, cytb, rpoB, clpC	[39]

Table 1. Cont.

Sarcocystis Species	Snake Species (Family)	Intermediate Host	Sarcocyst Description/Animal Tissue	Place of Sampling	Molecular Marker	Reference
<i>S. pantherophisi</i>	<i>Pantherophis alleghaniensis</i> <sup>a</sup> (Colubridae)	KO <i>Mus musculus</i>	Up to 3500 × 70 μm, smooth thin wall (<1 μm), protrusions 100 × 100 nm, GS 500 nm, bradyzoite 7.1–8.4 × 1.5–2.9 μm/tongue, abdomen, limbs, and masseter muscles.	Maryland, U.S. <sup>a</sup>	18S rDNA, 28S rDNA, <i>Cox1</i> , ITS1	[38]
<i>S. zuoi</i>	<i>Elaphe carinata</i> (Colubridae)	<i>Rattus norvegicus</i> <sup>a</sup>	2325–700 × 225–81 μm, striated wall, type 17, protrusion 10.2–12.5 × 2.0–2.5 μm, GS 0.4–0.6 μm, bradyzoite 7–10 × 2.6–3.0 μm/skeletal muscle through [54] the body, tongue, esophagus, and diaphragm 3000 × 0.9 μm, smooth thin wall (0.6–0.8 μm), type 1b, bradyzoite 6–8 × 1–1.5 μm, protrusions up to	China <sup>a</sup>	18S rDNA, ITS1, ITS2	[45,58]
<i>S. atheridis</i>	<i>Atheris nitschei</i> <sup>a</sup> (Viperidae)	<i>Mus musculus Lemniscomys barbarus</i>	150 × 90 nm, GS 0.6 ± 0.8 μm/tongue, triceps brachii, m. quadriceps femoris, abdominal wall, labium superior and inferior, masseter, diaphragm (one specimen)	Uganda, Africa <sup>a</sup>	18S rDNA	[33,59]
<i>S. gerbilliechis</i>	<i>Echis coloratus</i> <sup>a</sup> (Viperidae)	<i>Gerbillus gerbillus</i> <i>Gerbillus perpallidus</i> <i>Psammomys obesus</i> <i>Pachyuromys duprasi</i> <i>natronensi</i>	Up to 11.7 mm–190 μm, smooth, thin wall, type 1b, protrusions 80–180 × 50–120 nm, GS 300–1500 nm/esophagus, tongue, diaphragm, and thoracic and abdominal wall muscles	Galala mountain, Egypt <sup>a</sup>	No data	[34]
<i>S. muriviperae</i>	<i>Vipera palaestinae</i> <sup>a</sup> (Viperidae) <i>Coluber jugularis</i> (Colubridae) <i>Zamenis longissimus</i> <sup>a</sup> <i>Z. scalaris</i> <i>Elaphe quatuorlineata</i> <i>E. dione</i> <i>E. taeniura</i> <i>Pantherophis. obsoleta</i> <i>P. guttata</i> (Colubridae) <i>Crotalus scuiulatus</i> <i>scutulatus</i> <sup>a</sup> (Viperidae)	<i>Mus musculus</i>	5–8 mm × 150–400 μm, thick wall (3 μm), type 18b, bradyzoite 12–13 μm, protrusions ~3.5 μm/skeletal muscles	Tel Aviv, Israel <sup>a</sup>	No data	[35–37]
<i>S. clethrionomyelaphis</i>	<i>Z. scalaris</i> <i>Elaphe. quatuorlineata</i> <i>E. dione</i> <i>E. taeniura</i> <i>Pantherophis. obsoleta</i> <i>P. guttata</i> (Colubridae) <i>Crotalus scuiulatus</i> <i>scutulatus</i> <sup>a</sup> (Viperidae)	<i>Microtus arvalis</i> <i>M. oeconomus</i> , <i>M. guentheri</i> <i>Clethrionomys glareolus</i> <i>Eothenomys miletus</i> <sup>b</sup>	1200–4500 × 70–170 μm, smooth wall, type 9, protrusions ~3 μm, bradyzoites 10–12 × 2 μm/skeletal muscle (thigh, loin, rump, ribs), tongue, esophagus and diaphragm	Germany <sup>a</sup> Yunnan Province, China <sup>b</sup>	18S rDNA	[46,47,60]
<i>S. crotali</i>	<i>Crotalus scuiulatus</i> <i>scutulatus</i> <sup>a</sup> (Viperidae)	<i>Mus musculus</i>	4000 × 500 μm, smooth thin wall, type 1a, homogeneous GS (1 μm)/skeletal muscle of abdomen and flanks	California, U.S. <sup>a</sup>	No data	[25]
<i>S. hoarensis</i>	<i>Bittis nasicornis</i> <sup>a</sup> <i>B. arietans</i> <i>B. caudalis</i> <i>B. gabonica</i> (Viperidae)	<i>Meriones unguiculatus</i> <i>Mesocricetus auratus</i> <i>Phodopus sungorus</i> <i>Gerbillus perpallidus</i> <i>Mastomys natalensis</i> <i>Mus musculus</i>	2500 μm, secondary cyst wall, type 42, bradyzoite 11–12 × 2 μm/connective tissue (snout, tongue), subcutis and connective tissue (ear, scrotum, anus, base of the tail and soles of the feet)	East Africa <sup>a</sup>	No data	[30,32]
<i>S. zamani</i>	<i>Python reticulatus</i> <sup>a</sup> (Pythonidae)	<i>Rattus norvegicus</i>	1000–2000 × 300–500 μm, smooth thick wall (1–3 μm), type 18c, bradyzoite 10–12 × 1.5 μm (living cyst), 7–8 × 1–1.5 μm (formalin-fixed)/skeletal muscle throughout the body, including the tongue, esophagus, vagina, and diaphragm	Singapore <sup>a</sup> Malaysia <sup>a</sup>	No data	[12]
<i>S. villivillosi</i>	<i>Python reticulatus</i> <sup>a</sup> <i>P. timorensis</i> , <i>Aspidites melanocephalus</i> <i>Python sebae</i> (Pythonidae)	<i>Rattus norvegicus</i> and others <i>Rattus</i> spp. <i>Bandicota</i> spp.	700–1100 × 70–90 μm, striated thick wall (<2 μm), type 22, bradyzoite 5.0 × 1.0 μm/skeletal muscles through the body	Singapore <sup>a</sup> Malaysia <sup>a</sup>	No data	[12,16]
<i>S. idahoensis</i>	<i>Pituophis melanoleucus</i> <sup>a</sup> (Colubridae)	<i>Peromyscus aniculatus</i> <sup>a</sup>	Up to 9400 × 800 μm, smooth thin wall (~1 μm), bradyzoite 6–7 × 2.0 μm/tongue, thorax, abdomen and hind limb	Idaho, U.S. <sup>a</sup>	No data	[42,43]

Table 1. Cont.

Sarcocystis Species	Snake Species (Family)	Intermediate Host	Sarcocyst Description/Animal Tissue	Place of Sampling	Molecular Marker	Reference
<i>S. murinotechis</i>	<i>Notechis ater</i> <sup>a</sup> (Elapidae)	<i>Rattus norvegicus</i> , <i>R. lutreolus</i> [57] <i>R. rattus</i> <i>Pseudomys higginsi</i> <i>Mastacomys fuscus</i>	300 × 60–90 µm, thick wall, protrusion 6 × 4 µm/tongue, diaphragm and skeletal muscle	Southeastern Australia <sup>a</sup>	No data	[17]
<i>S. singaporensis</i>	<i>Python reticulatus</i> <sup>a</sup> <i>P. timorensis</i> , <i>Aspidites melanocephalus</i> <i>Python sebae</i>	<i>Rattus norvegicus</i> and others <i>Rattus</i> spp. <i>Bandicota</i> spp. <i>Nesokia indica</i> <i>Arvicantis niloticus</i>	Up to 4400 µm, striated thick wall (3–5 µm), type 19, bradyzoite 4.5–6 × 1.0–1.5 µm/muscles throughout the body, including tongue, neck, and appendages, diaphragm, trunk and skin muscles, striated muscles of the eye, esophagus, scrotum and vagina	Malaysia <sup>a</sup> Singapore <sup>a</sup> [6]	18S rDNA, ITS1, ITS2, 28S rDNA	[10–12,14,16,61]
<i>S. nesbitti</i>	<i>Python reticulatus</i> <sup>c</sup> (?) <i>Simalia amethystina</i> <sup>d</sup> (Pythonidae) <i>Naja kaouthia</i> <sup>c</sup> (?) (Elapidae)	<i>Macaca mulatta</i> <sup>a</sup> <i>Macaca fascicularis</i> <sup>b</sup>	Up to 2000 µm, smooth thin wall (0.46–0.60 µm), type 1, GS 0.5–0.76 µm, bradyzoite 7.5–10 × 1.25 µm/cervix, scapular region, abdomen, psoas, and diaphragm muscle	North India <sup>a</sup> Malaysia <sup>c</sup> Indonesia <sup>c</sup> Australia <sup>d</sup> China <sup>b</sup>	18S rDNA	[13,20,22,62,63]
<i>S. dirumpens</i>	<i>Bittis nasicornis</i> <sup>a</sup> <i>B. arietans</i> <i>B. caudalis</i> <i>B. gabonica</i> (Viperidae)	<i>Meriones unguiculatus</i> <i>Mesocricetus auratus</i> <i>Phodopus sungorus</i> <i>Gerbillus perpallidus</i> <i>Mastomys natalensis</i> <i>Mus musculus</i> <i>Grammomys caniceps</i> <i>Lemniscomys barbarous</i>	Up to 25,000 × 50–100 µm, smooth thin wall, type 1b, GS 0.5–1 µm, bradyzoite 8–9 × 2 µm/abdominal wall	East Africa <sup>a</sup>	No data	[31,64,65]

<sup>a, b, c, d</sup>: Equal letters mean that the naturally infected animals from which *Sarcocystis* sp. was isolated inhabit the same region where they were captured or sampled. Experimental hosts are not labeled with superscribed letters. Sarcocyst description: the word “type” refers to the 82 revised and/or proposed distinct types of sarcocyst walls [1].

Table 2. Snakes as definitive hosts of *Sarcocystis* spp. and lizards as their intermediate hosts.

Sarcocystis Species	Snake Species (Family)	Intermediate Host	Sarcocyst Description/Tissue	Place of Sampling	Molecular Marker	Reference
<i>S. acanthocolubri</i>	<i>Spalerosophis diadema</i> (Colubridae)	<i>Acanthodactylus boskianus</i> <sup>a</sup> <i>A. sculentu</i> <sup>a</sup> <i>A. paradalis</i> <sup>a</sup>	10,120 × 950 µm, striated thick wall (3.9 µm), protrusions 2.55 × 1.41 µm, GS 0.37 µm/fore-limb, hind-limb subcutaneous tissue and tail	Giza, Cairo, Baltem, Rashid, Marsa-Matrouh, Beni-suef, Egypt <sup>a</sup>	No data	[56]
<i>S. ameiva-mastigodryasi</i>	<i>Mastigodryas bifossatus</i> (Colubridae)	<i>Ameiva ameiva</i> <sup>a</sup>	1000–2000 × 80 µm, smooth thin wall, bradyzoite 7.2 × 2.5 µm/tongue, tail and hind limb	Pará, Brazil <sup>a</sup>	No data	[57]
<i>S. stenodactylicolubris</i>	<i>Coluber najadum</i> <sup>a</sup> <i>Coluber rogersi</i> (Colubridae)	<i>Ptyodactylus guttatus</i> <i>Stenodactylus grandiceps</i>	175–200 × 35–50 µm, thick wall (2 µm), type 4, protrusions up to 800 × 200–250 nm, bradyzoite 5–6 × 1.5 µm, GS 200–300 nm/tail, limbs, tongue	Syria <sup>a</sup>	No data	[50]
<i>S. lacertae</i>	<i>Coronella austriaca</i> <sup>a</sup> (Colubridae)	<i>Podarcis muralis</i>	Up to 640 × 230 µm, striated thick wall (1 µm), type 37a, bradyzoite 6.6 × 2.5 µm, GS 0.5–2.0 µm/tail 60–100 × 200–900 µm, striated wall (2–6 µm), type 9d, protrusions 1.9–3 µm, bradyzoite 4.1–5.3 × 0.8–2.0 µm/tail, hind limb and different parts of the body	Čabrad', Slovak Republic <sup>a</sup>	18S rDNA	[52,66,67]
<i>S. gongyli</i>	<i>Spalerosophis diadema</i> (Colubridae)	<i>Chalcides ocellatus ocellatus</i> <sup>a</sup>	4.1–5.3 × 0.8–2.0 µm/tail, hind limb and different parts of the body	Giza, Egypt <sup>a</sup>	No data	[55,68]

Table 2. Cont.

Sarcocystis Species	Snake Species (Family)	Intermediate Host	Sarcocyst Description/Tissue	Place of Sampling	Molecular Marker	Reference
<i>S. chalcidicolubris</i>	<i>Coluber raevergieri</i> <i>C. viridiflavus</i> <i>C. gemonensis</i> <i>C. najadum</i> (Colubridae)	<i>Chalcides</i> spp. <sup>a</sup>	124–275 × 27–53 μm, smooth thin wall, type 1e, protrusions 2 × 1 μm, bradyzoite 10–12 × 2 μm/tail	Israel <sup>a</sup>	No data	[49]
<i>S. podarcicolubris</i>	<i>Coluber viridiflavus</i> <sup>a</sup> <i>C. gemonensis</i> <sup>a</sup> Balkan whip <i>C. najadum</i> <i>C. jugularis</i> <i>C. raevergieri</i> <i>Macroprotodon cucullatus</i> (Colubridae)	<i>Lacerta</i> spp. <i>Podarcis</i> spp. <i>Algyroides nigropunctatus</i>	430–1300 × 90–290 μm, striated wall, type 9 m, protrusions up to 5 μm, bradyzoite 7.7–10 × 2 μm/tail's base and hind limb	Sardina <sup>a</sup> Sicily <sup>a</sup> Jugoslavia <sup>a</sup> Turkey	No data	[44,48,51,69]

<sup>a</sup>: Equal letters mean that the naturally infected animals from which *Sarcocystis* sp. was isolated inhabit the same region where they were captured or sampled. Experimental hosts are not labeled with superscribed letters. Sarcocyst description: the word "type" refers to the 82 revised and/or proposed distinct types of sarcocyst walls [1].

Published reports of unnamed *Sarcocystis* spp. from snakes are available in numerous databases; nonetheless, besides the detection of the parasite, a few have additional information such as morphologic assessment, molecular and phylogenetic analysis, or experimental transmission tests [27,29,70–72].

In South America, most studies about *Sarcocystis* spp. in snakes correspond to general parasitic surveillance in snakes without further tests for species identification [26,28]. The presence of sporocysts in the feces of two specimens of common boas (*Boa constrictor constrictor*) and one rainbow boa (*Epicrates cenchria cenchria*) were reported in the north of Brazil. No additional tests were performed [57]. Recently, sporocysts shed by a single specimen of *B. constrictor* were detected and molecularly characterized in the northeast of Brazil [72]. Based on 18S rDNA and *Cox1* markers, the isolate obtained from the *B. constrictor* is similar to *Sarcocystis* sp. isolated from the rodent plains viscacha (*Lagostomus maximus*) in Argentina [73]. As this Argentinian rodent species does not occur in the northeast of Brazil, it is possible that a different species of rodent acts as the IH of this *Sarcocystis* sp. derived from *B. constrictor*.

Although molecular analysis is mandatory to discriminate *Sarcocystis* spp., this methodology alone is not sufficient for a definitive diagnosis of a snake as the DH of the parasite. The multiplication of the parasite in the intestinal tract of a snake should be confirmed in order to prove that the shed sporocysts were indeed generated in the snake; a snake may shed sporocysts that already existed in any preyed animals. However, molecular evidence of infection indicates which *Sarcocystis* species may occur in that area [22,23,53,74] and may help to direct further investigations.

#### 4. Snakes as Intermediate Hosts of *Sarcocystis* spp.

Based on the prey–predator life cycle of *Sarcocystis* sp. [1], and the fact that some species of snakes are preyed on by other animals, snakes might serve as the IH for this coccidian. Some mammals, birds of prey such as Falconiformes, and reptiles (including other snakes) are known to prey on snakes [75–77]. Although reports on *Sarcocystis* sp. infecting snakes focus their role as DHs [1], a few studies show snakes as IHs of the parasite [78–80]; in all of these reports, the DHs remain unknown (Table 3).

**Table 3.** Snakes as intermediate hosts of *Sarcocystis* sp. and with unknown definitive hosts.

<i>Sarcocystis</i> Species	Snake Species	Sarcocyst Description/Tissue	Natural Habitat/Place of Sampling	Molecular Marker	Reference
<i>Sarcocystis moreliae</i>	<i>Morelia</i> cf. <i>viridis</i>	1.118 × 123 µm, smooth thin wall (0.55 µm), type I, bradyzoites 663,799 × 1.43–1.87 µm (LM)/head, trunk and tail	Indonesia/Czech Republic	18S rDNA, 28S rDNA, ITS1, <i>cox1</i>	[81]
<i>Sarcocystis</i> sp.	<i>Crotalus durissus terrificus</i>	up to 2000 × 50–150 µm, thin wall (<1 µm), type I wall, minimal GS, bradyzoite 1–2 µm, (LM; TEM)/Tongue, head, skeletal muscle adjacent to vertebra	South America/Tennessee, USA	18S rDNA	[82]
<i>Sarcocystis atractaspidis</i>	<i>Atractaspis leucomelas</i>	up to 7 × 4 mm	Kenya/Kenya	No data	[80]
<i>S. pythonis</i>	<i>Python spilota</i>	up to 1100 µm, thin wall, bradyzoite 4 × 7 µm		No data	[79]

Sarcocyst description: the word “type” refers to the 82 revised and/or proposed distinct types of sarcocyst walls [1].

The first report of the presence of sarcocysts in snakes was in an Australian carpet snake or Australian Python (*Morelia argus*). This species was named *Sarcocystis pythonis*. It was described as having a thin-walled cyst with a type I wall [79]. The other species, *Sarcocystis atractaspidis*, was identified later from two burrowing vipers (*Atractaspis* sp.). In this case, sarcocysts were observed in the mesentery and lungs and were macroscopic [80]. In both reports, these species were considered *inquirendae*, because no visual evidence (images) was reported in these papers, besides the lack of TEM analysis, differential morphological descriptions and molecular tests [1,82].

Through molecular analysis, *Sarcocystis* sp. was incidentally detected in tissues of the snakes *Hemorrhoids* sp. and *Malpolon* sp. during an investigation of *Hepatozoon* sp. using the 18S rDNA gene. GenBank screening indicated that the closest match was *S. lacertae*, which uses snakes as the DH and lizards as the IH [78]. In the following year, sequences of 28S rDNA gene were reported from the *Sarcocystis* sp. of a *Psammophis* sp. snake in the Mediterranean region [83]. A phylogenetic analysis was performed, but the obtained results did not clarify the prediction of the DH; the obtained nucleotide sequences did not form a monophyletic group with other *Sarcocystis* infecting snakes, and the available sequences were placed in unrelated groups [83]. A number of studies indicate that natural *Sarcocystis* spp. groups are mainly influenced by their DH correlations, as opposed to their relationships with IHs [84,85]. Molecular evidence of *Sarcocystis* sp. in tissues of the crowned leafnose snake (*Lytorhynchus diadema*) was reported, although no phylogenetic analysis was conducted [86]. In these three studies, only molecular evidence was shown for *Sarcocystis* spp. infecting snakes' tissues.

A South American rattlesnake (*Crotalus durissus terrificus*) was confiscated from an illegal owner in Tennessee. The animal died in custody and a necropsy was performed. Macroscopically, no lesions or cysts were observed in the animal's tissues. Under light microscopy, cysts measuring up to 2000 µm in length were visualized in the skeletal muscles from three areas near the vertebrae, as well in the tongue and head. The sarcocysts' wall was thin and classified as type I. At the time, there was no evidence of active merogony. The authors considered the cyst morphology distinct from the usual sarcocyst form in some aspects, leading them to hypothesize the possibility of the snake being an aberrant IH [82].

A molecular analysis of *Sarcocystis* sp. from the South American rattlesnake demonstrated a high similarity with *Sarcocystis mucosa* isolated from Australian marsupials; this finding led the authors to hypothesize that an opossum species from South America, which is also a marsupial and shares the same native habitat as this snake species, could be its DH. However, the nucleotide sequence of 18S rDNA obtained in this study was relatively short, and no phylogenetic test was performed. Other animals have been suggested as DHs based on their food habits, but this remains unclear [82]. In another study, 99% identity was found for *L. diadema* isolate and *Sarcocystis* sp. from the Virginia opossum (*Didelphis*

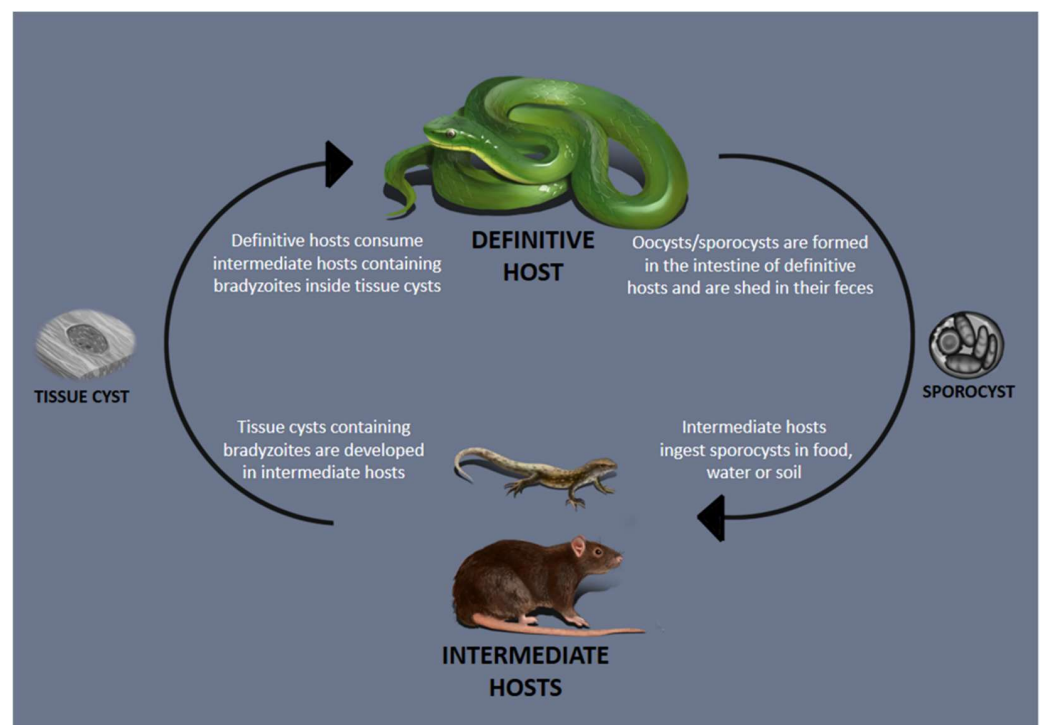


*virginiana*) from Mexico and *Sarcocystis neurona* from fisher (*Martes pennanti*). Thus, it was suggested that the probable DH could be an omnivorous or carnivorous mammal [86].

Recently, the presence of *Sarcocystis* sp. in the head, trunk and tail muscles of the green tree python (*Morelia cf. viridis*) has been reported [81]. This parasite was classified as a new species and was named *Sarcocystis moreliae*, based on morphological and molecular characteristics. This isolate differs from the one found in *C. d. terrificus* in terms of size and cyst wall thickness. Molecular analyses successfully generated sequences of the 18S rDNA, 28S rDNA, ITS1, and *cox1* loci. Despite obtaining robust sequences, the comparison with other species that infect reptiles as IHs was limited due to the lack of genetic information, such as for the ITS-1 region or very short sequences available. The absence of morphological information also hampered the comparison between species. Phylogenetic analysis could not predict the DH of the species. Nevertheless, the authors proposed that the DHs of this species might include monitors, birds, birds of prey, quolls, or even other snakes [81].

### 5. Intermediate Hosts of *Sarcocystis* spp. from Snakes

Serpents have a wide range of animals in their food spectrum, including mammals, birds, reptiles and amphibians, depending on the snake species, life habits and body size [87,88]. Nonetheless, only mammals and reptiles have been recorded as IHs of *Sarcocystis* spp. which use snakes as DHs [1,38,39] (Figure 1). We hypothesize that birds that commonly feed on the soil are more exposed to the ingestion of sporocysts and possibly serve as IHs for certain species of *Sarcocystis* that cycle in snakes. *Sarcocystis* spp. that have mammals as IHs are more numerous when compared to those that use reptiles, embracing 16 out of 23 species [1,38,39]. Initial descriptions of *Sarcocystis* sp. were mainly based on sarcocysts' morphology [31,48,51]. However, the morphology of sarcocyst walls is not reliable because some walls may be almost identical among certain species and may be affected during sample processing or may be changed with age [89]. Molecular analysis is a valuable and necessary resource for the classification of *Sarcocystis* on the species level, besides morphological and biological aspects of the parasite and animal hosts [3,90,91].



**Figure 1.** Life cycle of *Sarcocystis* spp. involving snakes as definitive hosts. Their intermediate hosts encompass distinct species of small mammals and lizards.

Rodents comprise the largest order in the class Mammalia, encompassing more than 2000 animal species and almost 500 genera [92]. *Sarcocystis* spp. of snakes are reported to infect around 20 genera of rodents (Table 1). *Sarcocystis* spp. shed by snakes are less specific to their IHs than to the DHs. *Sarcocystis singaporensis*, for example, infects at least 12 IHs of the genus *Rattus* and *Bandicota* [16]. *Sarcocystis dirunpensis* and *S. hoarensis* infect six different animal genera belonging to two families: Muridae and Cricetidae [31]. Exceptions are observed as well, and a single animal species acts as IH for *S. muriviperae* [37]. Other *Sarcocystis* species that demonstrate specificity to their IHs are *S. idahoensis*, *S. zamani*, *S. zuoi*, *S. pantherophis*, *S. crotali* and *S. attenuati*; however, with exception of *S. idahoensis*, experimental infections were not performed for the remaining *Sarcocystis* spp., or were conducted with a limited range of potential IHs for these *Sarcocystis* spp. Moreover, attention must be given to *S. pantherophis*, since the only IH is an experimental immunosuppressed mouse (KO *Mus musculus*); thus, the putative IH could be a different murid. Although *S. gerbilliechis* has also been shown to have low IH specificity on the genus and species levels, only members of the subfamilie Gerbillinae were demonstrated to be IHs of the parasite under experimental infections [34].

The genus *Rattus* is reported to be an IH for *S. zuoi*, *S. zamani*, *S. villivilosus*, *S. murinotechis* and *S. singaporensis*. Out of these species, *S. murinotechis* and *S. singaporensis* stand out due to their pathogenicity to their IH. *Sarcocystis singaporensis* is one of the most studied *Sarcocystis* spp. of snakes [93] and this species is highly lethal to different species of *Rattus* sp. and to the short-tailed bandicoot rat (*Nesokia indica*) [14]. Due to its pathogenicity to IH, *S. singaporensis* was tested as an efficient biocontrol agent for non-native rats [14,15,94].

Voles serve as IHs for *S. clethrionomyelaphis* [46,47,60]. Initially, only the genera *Microtus* and *Clethrionomys* were described as IHs for *S. clethrionomyelaphis*, and, at that time, these animals were classified as IHs under experimental infections [46]. Years later, the large oriental vole (*Eothenomys miletus*) was identified as a natural IH of *S. clethrionomyelaphis* through morphology and molecular analysis [47]. This vole is endemic to Yunnan Province, China [95], and the frequency of *S. clethrionomyelaphis* infection in these animals was 75.8% [47], demonstrating that this *Sarcocystis* species is spread in the region. Recently, a new species was classified as *S. attenuati* from the Asian gray shrew (*Crocidura attenuata*) in the same region where *S. clethrionomyelaphis* was found; morphological and molecular features were employed to differentiate *S. attenuati* from *S. clethrionomyelaphis*. Still, different from the other *Sarcocystis* spp., sarcocysts from *S. attenuati* were also found in the heart of the IH [39].

Intermediate hosts of snakes' *Sarcocystis* spp. are more susceptible to developing clinical signs than the DHs. In contrast to reptiles as IH, rodents have been demonstrated to be more susceptible to developing a pathology caused by *Sarcocystis* spp. The occurrence of lesions is induced by early stages of the parasite and is associated with the immune response of the host and the dose of parasites [1,33]. In most *Sarcocystis* spp. that cause acute sarcocystosis, the pathology is associated with damage in the liver. This may be observed in *S. idahoensis*, *S. muriviperidae* and *S. atheridis*, where meronts were observed in hepatocytes. Hepatosplenomegaly, anorexia, weakness and icterus are some of the clinical signs commonly observed [33,37,42]. The pathology is so severe that it can lead the animal to death. Specificity is also noted in the pathogenicity level. The same infective dose of *S. atheridis* was given to both IHs of this species; nevertheless, only the barbary striped grass mouse (*Lemniscomys barbarus*) was demonstrated to be susceptible to disease. *Sarcocystis gerbilliechis* also cause hepatopathy in their IHs; however, the lesions are focused in the liver parenchyma and endothelial cells of other organs and usually the animals survive to the acute signs [34].

Other pathologies reported in IHs of *Sarcocystis* spp. from snakes are neurological and respiratory alterations. Meronts of *S. pantherophisi* were seen in the cerebellum of gamma-interferon gene knockout mice (*Mus musculus*) and were related to clinical signs observed in these animals. Early stages of the parasites *Sarcocystis murinotechis* and *S. singaporensis* can be found in the endothelium of different tissues through the body; however, lungs are the most infected tissue, leading to severe respiratory alterations and culminating in death [10,12,17]. In both species, the induction of edematous lungs and effusion were observed, but hemorrhage was only reported for *S. singaporensis*. Dyspnea was observed in IHs susceptible to the disease caused by *S. singaporensis* [14].

Molecular diagnosis and phylogenetic analysis results may predict the DH for certain *Sarcocystis* spp. Some sarcocysts found in rodents were identified and classified even without knowledge of their DH. Through phylogenetic data it is possible to assume the suitable DH, as observed for *S. eothenomysi* and *S. scandentiborneensis* [96,97].

Reptiles from the Saurian families Lacertidae, Scincidae and Gekkonidae are known to be IHs for seven species that have snakes as DHs; these species are as follows: *S. podarcolubris* [48], *S. gongyli* [68], *S. stenodactylicolubris* [50], *S. chalcidicolubris* [49], *S. acanthocolubri* [56], *S. ameivamastigodryasi* [57], *S. lacertae* [66] (Table 2).

*Sarcocystis podarcolubris* was the first species found to exhibit a life cycle involving two reptiles, with a lizard being the IH [48]. Initially, two species from the genus *Podarcis* were described as IHs following experimental infections [48]. In the same paper, a reference was made to a report on a different species of *Podarcis* sp., sharing close characteristics with *S. podarcolubris*; however, this species was not formally named. Subsequently, experimental transmission defined the genera *Lacerta*, *Podarcis* and *Algyroides* as additional IHs for *S. podarcolubris* [51]. Regarding the extensive range of IHs for reptilian sarcosporidia, other lizard genera that could be infected by *S. podarcolubris* were tested, and fortuitously a distinct sarcocyst was detected in the musculature of *Chalcides* spp. (eyed skink, *Chalcides ocellatus*; Gran Canaria skink, *Chalcides sexlineatus*; West canary skink, *Chalcides viridanus*). A new species was postulated and designated as *Sarcocystis chalcidicolubris*, based on observations made through light and electron microscopy, as well as on the biology of the parasite [49].

Trinci [68] described a cyst found in the muscles of an eyed skink (*Chalcides ocellatus*) and named it *Sarcocystis gongyli*. Years later, numerous sarcocysts in the muscles of the skink from a different genus were described, although the DH was not defined [98]. Posteriorly, a study revealed the presence of sarcocysts in skeletal muscles of a native skink (*Chalcides ocellatus*) from Egypt, and through light and transmission electron microscopy, these sarcocysts were identified according to Trinci's classification (*S. gongyli*) [55]. Despite sharing the same IH, *S. gongyli* and *S. chalcidicolubris* were proved to be distinct species based on differences in the sarcocyst's morphology and experimental hosts.

Babudieri [66] re-described a species initially found by Lühe [99] in a common wall lizard (*Podarcis muralis*) and designated it *Sarcocystis lacertae*; nonetheless, there was no evidence of its DH. Sporocysts were isolated from a colubrid snake, the smooth snake (*Coronella austriaca*); then, experiments were conducted to identify its possible IH, and *Podarcis muralis* was the only species that became infected [52]. As *S. podarcolubris* shares the same IH as *S. lacertae*, it could potentially be the same species, but the life cycle trials demonstrated specificity related to the DH, and certain morphological differences kept them classified as separate species [48,52].

Chronologically, until the 1990s, only lizards from the families Lacertida and Scincidae had been identified as IHs for *Sarcocystis* species with snakes as DHs [44,48,55]. *Sarcocystis stenodactycolubris* was the first identified species that uses geckos as IHs [50]. *Sarcocystis* had been found in gekkonids skeletal muscles [100–103], and were classified as *Sarcocystis platydactyli* [104]. However, the DH of this species remains unknown to date. Modry et al. [50] isolated sporocysts from a Colubridae snake and performed experimental infections using six different lizard and two rodent species. Only geckos (Fan-fingered gecko, *Ptyodactylus guttatus*, and Jordan short-fingered gecko, *Stenodactylus grandiceps*) were found to be infected and sarcocysts were observed in tail, limb and tongue muscles.

The giant ameiva or Amazon racrunner (*Ameiva ameiva*) was identified as the IH of *S. ameivamastrigodryasi* through both natural and experimental infections. This *Sarcocystis* species is the only one with a snake–reptile cycle classified in the Americas until now [57]. *Sarcocystis acanthocolubri* is currently the most recent species known to have snakes as DHs and other reptiles as IHs [56]. The assessment revealed two differently sized cysts in all the studied species (Bosc’s fringe-toed lizard, *Acanthodactylus boskianus*; Nidua fringe-toed lizard, *Acanthodactylus sculentus*; and leopard fringe-fingered lizard, *Acanthodactylus pardalis*). However, it was demonstrated that they belong to the same species [56]. All *Sarcocystis* spp. detected in other reptiles as IHs were classified solely based on morphological and biological findings through natural or experimental infections. Molecular screening would elucidate whether these species are unique or not, and would help to identify other species.

## 6. Infection in Humans by *Sarcocystis nesbitti*

A few *Sarcocystis* species are known to infect humans and, in some cases, are capable of causing disease [19,20,105]. Humans serve as DHs or IHs in the life cycle of certain *Sarcocystis* spp. They act as DHs after consuming mature sarcocysts present in undercooked meat [89]. Usually, humans do not develop any symptoms and remain asymptomatic. But in certain cases, this intestinal sarcocystosis culminates in a gastrointestinal condition that is mostly self-limited and bland [105]. When humans ingest water or food contaminated with sporocysts, they might develop muscular sarcocystosis. In such cases, they may experience some symptoms such as fever, myalgia, headache, fatigue and arthralgia [19,20,89,106].

Early descriptions of *Sarcocystis* spp. in humans had been based on the sarcocyst wall morphology [89]. However, this method of classification and species distinction is questionable, as mentioned before. More than 100 studies in Europe, Latin America, Africa and Asia have reported sarcocysts in human tissues [19,20,89,107,108]. Most of the reported cases are concentrated in Southeast Asia. Some of these cases had been published a long time ago as isolated occurrences, and the diagnosis was based on symptoms, epidemiology and, in some cases, by biopsy findings. During that time, no species had been truly identified, but sarcocysts were morphologically described.

Southeast Asia has been known as an endemic region for acute muscular sarcocystosis (AMS) [89,109]. However, epidemiological surveillance of human AMS in this region is considered scant [19]. In a study in Malaysia, the prevalence of 21% of *Sarcocystis* spp. cysts was found in 100 humans’ tongues [18]. The samples were obtained from autopsies in a university hospital in Malaysia. A survey for sarcocysts in more than 1500 samples of limb muscles was undertaken in the same institute and no sarcocysts were observed (unpublished data) [20]. A seroprevalence survey was also carried out [110]; however, serology does not allow a distinction between muscular and intestinal sarcocystosis [111].

In the 1990s, an outbreak of human AMS was reported involving militaries from the U.S. that were in Malaysia for work. In this report, seven out of fifteen individuals exhibited similar symptoms in some degree, but only one case was confirmed through biopsy. The other patients were assumed to have AMS based on the common exposure, symptoms and laboratorial changes [106]. In the past decade, a series of outbreaks of AMS in Tioman Island and Pangkor in Malaysia were published, leading to an in-depth investigation with many affected people [19,20,23,109]. One of the biggest outbreaks to happen in Southeast Asia was a case involving 100 individuals in different periods between 2011 and 2012; most of them were travelers from Europe, and the initial diagnosis was based on clinical and laboratorial patterns of myositis. A biopsy was performed in eight patients, with six showing typical histological myositis, and sarcocysts were visualized in two cases [19]. In 2012, another outbreak was reported involving 89 symptomatic individuals who had visited Pangkor Island [20,109]. In this outbreak, sarcocysts were visualized through biopsy and the causative agent was identified by PCR; the 18S rDNA sequences were from *Sarcocystis nesbitti*.

Later, a previously reported AMS outbreak [19] was re-described [112], extending the study period to 2013 and considering 62 out of 99 cases as probable AMS with 6 confirmed cases. They performed a molecular analysis on a single sample of a patient accessing the 18S rDNA gene and identified *S. nesbitti* as the causative agent of the infection. In both cases [20,112], the authors presumed that all symptomatic patients were probably infected with the same species, given their shared location, similar travelling habits and symptoms. In recent years, a confirmed case of *S. nesbitti* was reported involving one Vietnamese person who traveled to Malaysia [108]. Humans are known to be IHs of *S. nesbitti*. Nevertheless, humans are reported as accidental hosts, because they do not have any natural predators to move the cycle forward [89,113].

*Sarcocystis nesbitti* was first mentioned in 1969 by Mandour [62], who found a different cyst in the muscles of two Rhesus monkeys (*Macaca mulatta*) from North India. However, the validity of *S. nesbitti* was questioned [63,114] due to the lack of TEM analysis, the absence of photomicrographs and the controversial description of cyst morphology. Afterward, sarcocysts were identified in the musculature of a long-tailed macaque (*Macaca fascicularis*) from a monkey farm in Yunnan Province, China. The sarcocysts were thoroughly described through LM and TEM, leading to their classification as a different species from *Sarcocystis kortei*, and then classified as *S. nesbitti* [63]. Based on ultrastructure, four other reports in monkeys [115–118] and two in humans [119,120] were mentioned as *S. nesbitti* by Yang et al. [63]. Consequently, it was assumed that *S. nesbitti* may infect other species of monkeys such as sooty mangabey (*Cercocebus atys*) and Guinea baboon (*Papio papio*) besides the genus *Macaca*.

A molecular analysis of *S. nesbitti* was performed years later by sequencing *Sarcocystis* sp. 18S rDNA derived from muscle samples of a long-tailed macaque [21]. Phylogeny demonstrated that these sequences were closely related to two *Sarcocystis* spp. that have snake–rodent life cycles, as well as one that was isolated from a racoon in Japan, but with origin in North America [121]. Therefore, it was hypothesized that snakes may be the DHs of *S. nesbitti*. The first indication of this assumption came from the molecular detection of this species in snakes' stools, although no oocysts/sporocysts were observed [22]. Phylogenetic analysis using the 18S rDNA region demonstrated that two sequences, one from the monocled cobra (*Naja kaouthia*) and the other from the reticulated python (*Phyton reticulatus*), were closely related to *S. nesbitti* [22]. The following year, a similar approach was implemented, and once again no oocysts/sporocysts were observed, but a sequence from the Indian cobra (*Naja naja*) was obtained and the isolate was placed near other isolate of *S. nesbitti* [21], along with two human isolates that were sequenced in this research [23].

Despite molecular evidence, the identification of oocysts/sporocysts in fecal samples is important to diagnose intestinal sarcocystosis [89]. Sporocysts were observed in the stool shed by the amethystine python (*Simalia amethystina*) in Australia, and presented 18S rDNA sequences with high similarity to *S. nesbitti*; phylogenetic analysis grouped them closely to *S. nesbitti* clades, including isolates of humans, monkeys and snakes. This finding confirms the earlier hypothesis of a snake as the DH of *S. nesbitti* [13]. Previous findings were concentrated in Southeast Asia, and the reticulated python was assumed to be the potential DH for *S. nesbitti*. Nonetheless, in the same study in which *S. nesbitti* was probably found in Australia, only *S. singaporensis* was detected in Indonesia, Malaysia, Thailand, the Philippines and Singapore [13]. Later, a molecular screening using 18S rDNA indicated *S. nesbitti* in reticulated python in Tioman Island [53]. As the first identified IH of *S. nesbitti* (macaque) does not occur in Australia, the authors [13] have questioned the snake–monkey cycle based on the diet of its potential predator, classifying this IH as accidental. Thus, they suggest rodents as potential IHs for the parasite. Further experiments associated with molecular analysis using additional markers rather than 18S rDNA are needed to respond to all the gaps involving *S. nesbitti*.

Regarding the source of *S. nesbitti* infection in humans in the past outbreaks, food and water were considered potential sources of infection [19,20,106]. Surveillance studies were conducted for the molecular detection of *Sarcocystis* sp. in water samples; however, the results were negative [20,122]. Molecular evidence of *S. nesbitti* was found in tank and river water from two villages located in Tioman, Malaysia. Although no oocysts/sporocysts were found, this study suggested that water was the main source of infection [123]. For this reason, it is important to develop preventive policies for both natives and tourists in Southeast Asia, focusing on water safety; it is recommended to drink boiled or bottled water, and to use this same type of water for activities such as brushing teeth, and to avoid uncooked food [20,123].

## 7. Molecular Diagnosis of *Sarcocystis* spp. of Snakes

Studies on the identification of sarcocystid organisms have been counting on the valuable contribution of molecular methods to resolve phylogenetic and taxonomic issues. Molecular identification has long been recommended as a mandatory approach for the complete description of a species, not only for the Sarcocystidae family [124–126], but also for other taxonomic groups [127]. Among the approximately 20 studies on the description of species of the genus *Sarcocystis* in snakes, those that were published before the 1990s had no molecular data, which has become an obligatory requirement since this period. Nowadays, the most used markers for the molecular identification of Sarcocystidae of the genus *Sarcocystis* are nucleotide sequencing nuclear ribosomal loci and loci encoding mitochondrial proteins, located in the mitochondrial genome [38,128–130].

Among the *Sarcocystis* species that infect snakes already described so far, there is information from molecular data for only ten of them, and partial sequences of the gene coding for the small subunit ribosomal RNA (18S rDNA) were provided for all these ten cases. Gene coding for 18S rDNA is a universally used molecular marker that is formed by variable regions interspersed with conserved regions, which allows the design of probes capable of amplifying organisms of different taxonomic levels by PCR. About 1800 nucleotides spanning nine helices, numbered V1 to V9, typically form the 18S coding sequence of *Toxoplasma gondii*, the most known Sarcocystid. Within 18S, the V4 region is a hypervariable and distinctive region among eukaryotes, rendering V4 the most used segment in sarcocystids [131]. The hypervariable V4 segment is present in 100% of the 18S rDNA sequences from *Sarcocystis* sp. identified in snakes that are available in Genbank.

Although superior in terms of phylogenetic informativeness for evolutionary studies [126], genes encoding the large subunit ribosomal RNA (28S rDNA) are much less frequently explored in studies on sarcocystids and, as a consequence, as well in studies on sarcocystids of snakes. Larger than 18S rDNA (28S is about 4000 nucleotides long in sarcocystidae), eukaryotic 28S rDNA sequences are also composed of conserved segments

interspersed with hypervariable segments [131]. The hypervariable domains of 28S rDNA are characterized by expansions of nucleotide sequences, numbered from D1 to D12, whose origin and function are not elucidated [131]. Typically, the segments usually identified in Sarcocystids are those with the greatest discriminatory power and include the D2 and D3 domains. From the three species of *Sarcocystis* of snakes that have been 28S rRNA gene sequenced, all of them encompassed the domains D2-D3.

A third marker located within the ribosomal locus used for the identification of species of the Sarcocystidae family are the transcribed internal spacers (ITS). These sequences do not encode known products and their function is unknown. The transcribed internal spacers are interregnum nucleotide sequences between the 18S rDNA and 5.8S ribosomal RNA coding genes, and between 5.8S and 28S rDNA, called ITS1 and ITS2, respectively [132]. Phylogenetic studies using ITS are less common, because it is not possible to use approaches based on structural alignment between homologous sequences, since they are not coding sequences and therefore the alignment of distant taxa is not very accurate [133,134]. However, ITS1 is a marker with the potential to discriminate between species with high similarity and for which differentiation with markers of lower evolutionary rate was not possible [130]. *Sarcocystis moreliae*, *Sarcocystis zuoi*, *Sarcocystis pantherophisi*, *Sarcocystis attenuati*, *Sarcocystis singaporensis* and *Sarcocystis zamani* were identified by both 18S rDNA and ITS1.

Information from non-nuclear genes such as those from the mitochondrial and apicoplast genomes has also been used to identify sarcocystids, but less frequently than the ribosomal locus genes. Among them, studies using cytochrome oxidase coding genes predominate. Cytochrome oxidase is an enzyme that participates in the electron transport chain in eukaryotes and is therefore involved in the cellular energy metabolism. This gene is present in all organisms that have mitochondria and hence is widely used as a marker in molecular phylogenies [127]. Although widely used in DNA barcoding studies, *cox1* has insufficient discriminatory power to identify species that use birds as IHs. This group of organisms can only be discriminated using ITS1 analysis, whereas they are almost identical regarding both the 18S rRNA gene and the D2/D3 domain of the 28S rRNA gene [130]. *Cox1* has been used to identify three species of snake-derived Sarcocystids: *Sarcocystis moreliae*, *Sarcocystis pantherophisi* and *Sarcocystis attenuati*.

## 8. Future Directions

For most reports of *Sarcocystis* spp. in snakes, no molecular data are available. Therefore, besides relevant biological and morphological aspects of the parasites and their animal hosts, it is crucial to provide genetic information for the observed *Sarcocystis* spp. This will favor the identification of potential hosts involved in their life cycles. When sporocysts or tissue cysts are detected in snakes, PCR and nucleotide sequencing of the most informative markers (ex. ITS1, *cox1*, 28S rDNA and 18S rDNA) are needed. Then, the sequencing matches may show putative hosts for the parasite.

If the molecular analysis of sporocysts detected in snakes does not provide any information on the potential IH involved in its life cycle, a bioassay may be an alternative approach. Since most reports of snakes acting as DHs use small mammals as IHs, initial attempts at bioassays should focus on frequently found small mammals inhabiting the same region as the snake. Small rodents such as rats and mice are good candidates in bioassays, as they are widely distributed in most geographic regions and laboratory rodents may be acquired for experimental purposes. Searching for lizards as IHs of *Sarcocystis* sp. from snakes is more challenging, as these animals are rarely raised for research or for human food.

For those snakes that act as IHs for certain species of *Sarcocystis*, their DHs are completely unknown to date. Future studies should be conducted to generate information about sarcocysts in snakes, including genetic characterization of the parasite. The search for potential DHs for these *Sarcocystis* spp. is also difficult, as these DHs may include several classes of predators, including mammals, birds and reptiles that may prey on snakes.

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