

The validity of *Sarcocystis sinensis*

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DEAR EDITOR:

Recently, in a publication of Dubey et al (2014) was suggested that *Sarcocystis sinensis* was a *nomen nudum* based on what they considered insufficient description of the parasite and lack of publicly available voucher material. They singled out that Zuo et al (1990) was not an appropriate venue for publishing a new species description, but we believe they did not adequately acknowledge two other reports in 1992 and 1995 when reaching their conclusion. Dubey et al (2014) correctly state that "the species of *Sarcocystis* from the water buffalo in China was presented under the name '*S. sinensis*' by Zuo (Zuo et al, 1990) at a national meeting (Fifth Symposium of the Chinese Society of Protozoology, Chongqing), and an abstract without figures was released." (Dubey et al, 2014). That presentation would not serve as a valid basis for naming the new species.

However, *S. sinensis* was first formally published by Zuo et al (1995). There, in the book "the Proceedings of the Tenth Anniversary of the Founding of the China Parasitological Society" (ISBN 7-5046-2012-2, Chinese Science and Technology Express, Beijing, China, 1995), and a book "Coccidians in Livestock and Birds and Human Coccidiosis" (ISBN 7-5308-1195-9, Science and Technology Publishing Company of Tianjin, Tianjin, China, 1992), the cyst's clear and detailed morphological structure was described and the life cycle was studied by Zuo (Zuo, 1992; Zuo et al, 1995). We present here the translation of the original descriptions as well as the detailed morphology of muscular cysts of *Sarcocystis sinensis* and infection experiments.

In the book (Zuo et al, 1995), the new species was described from cysts in muscles of water buffalo (*Bubalus bubalis*) from Kunming, Yunnan, China. Cysts were long and thin and/or fusiform in shape, 1 250 μm \times 100 μm (480-3 570 μm \times 45-152 μm) in average size. Leaning, finger-like protrusions (Figure 1, 10), 4.2-10.1 μm in size, averaged 5.8 μm , contained microtubules and few electron-dense granules (Figure 1, 11). There were invaginations on the surface of the protrusions' mostly in the middle and base parts. Ground substrate was 0.4-1.7 μm in thickness, extending inside of the cyst formed the septa-like structures or segmentation inside the cyst, in which a lot of concentrated banana-shaped bradyzoites, 13.0 μm \times 3.6 μm (10.9-16.1 μm \times 2.7-4.7 μm) in size could be seen. Under the scanning electron microscopy (SEM), the tip surface of the

protrusions was arranged as regular square-shaped structures with space measured 0.4 \times 0.8 μm , between reach other (Figure 1, 12). Natural prevalence was estimated at 58.0%. Location of parasite was skeletal muscle. Light microscopy (LM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were presented. The cysts structure under LM, TEM and SEM were presented from both books were copied as Figure 1.¹

In order to identify its definitive host, experimental infections were conducted initially in carnivorous animals. The first experiment was performed from April to May, 1988. Two dogs, two cats and two rhesus monkeys (*Macaca mulatta*) were infected with fresh *S. sinensis* cysts isolated from water buffalo. Each animal was fed with 60 cysts. Stools of the infected animals were examined for the presence of sporocysts and oocysts from day 5 to day 40 post feeding. Finally, animal were killed and the scrape from the intestinal mucosa was examined for *Sarcocystis* sporocysts and oocysts. No sporocysts and oocysts were found. Second experiment was performed from December 1989 to June 1990, and three cats, three dogs, one eurasian sparrow hawk (*Accipiter nisus*), a boreal owl (*Aegolius funereus*), an eurasian tawny owl (*Strix aluco*), and a little-banded goshawk (*A. badius*), two vultures (*A. monachus*) were infected with *S. sinensis* cysts. Each animal were fed with 200 cysts. Stools of the infected animals were examined for the presence of sporocysts and oocysts from day 5 to day 40 post feeding. Except of goshawk and vultures, all animals were checked as first experiment in 1988, killed and intestinal mucosa were examined. No sporocysts and oocysts were found. The third infection experiment was performed in 1995. Two volunteers (one 55 years old woman and one 29 years old man) swallowed 1 600 and 2 000 cysts respectively, but no sporocysts and oocysts were found later.

These attempts demonstrated that none of these hosts serves as the definitive host for this parasite (Zuo et al, 1995). The definitive host of *S. sinensis* remains unknown to this day.

The identification of *S. sinensis* is as following:

Intermediate host: water buffalo

Definitive host: unknown

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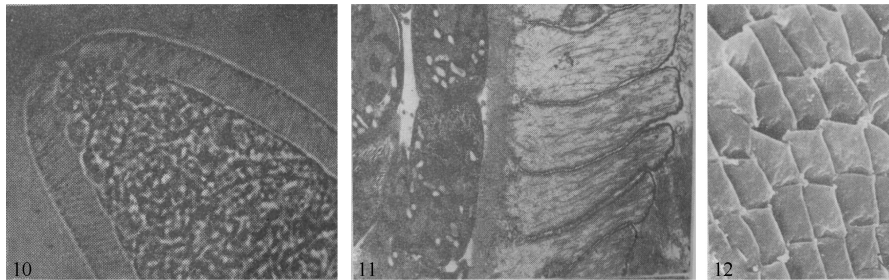


Figure 1 Cysts structure under LM, TEM and SEM presented from the book of Zuo et al (1995)

10: *S. sinensis* cyst from naturally infected water buffalo (1 400×, LM); 11: Cyst wall section of *S. sinensis*, showing the protrusions, microtubules few electron dense granules (7 000×, TEM); 12: Cyst surface protrusions of *S. sinensis* (10 000×, SEM).

Found place: Kunming, China

S. sinensis samples were stored at the museum of Department of Biology, Yunnan University, Kunming, China. The samples were cysts fixed in glutaraldehyde.

We consider that naming the new species as *S. sinensis* completely met the requirements of *The International Code of Zoological Nomenclature* (ICZN). The book (a collection) was published formally in 1995 (ISBN 7-5046-2012-2). It wasn't the 1985-1995 proceedings mentioned by Dubey et al (2014), but rather was an open publication to commemorate the China Society for Parasitology, founded a decade earlier. It was a peer-reviewed book. Its preface specified that only 93 studies were selected from more than 200 manuscripts submitted for publication. Eight new species from *Metahaliotrema*, *Protogyrodactylus*, *Bicotyle*, *Diplectanum*, *Diplectanum*, *Lamellodiscus*, *Pseudocryptotropa*, as well as *Sarcocystis* respectively were published. Although it is true that the ICZN stipulates that "materials issued primarily to participants at meetings (e.g. symposia, colloquia, congresses, or workshops) including abstracts and texts of presentations or posters" do not constitute a published work, we believe that Zuo (Zuo et al, 1995) fully meets the criteria required for describing a new species. We emphasize that Zuo's work was not published in collections of meeting proceedings from 1985-1995 mentioned by Dubey et al (2014) (although such an unpublished collection, in Chinese, does exist). Rather, this was a formally published in a book with an ISBN designation. The ICZN code does not stipulate that publishing a new species must take place in a journal. Moreover, article 8 of the ICZN code defines a published work as an edited material, which since origin is available and which constitutes a high number of identical copies of long term material. Two formally published books have been issued 2100 copies since published, and all of them were commercialized.

Some samples of *S. sinensis* were subsequently identified by investigators according to the initial morphological description. Photos were showed by several investigators who found *S. sinensis* (Chen et al, 2011; Jehle et al, 2009; Li et al, 2002; Moré et al, 2014; Yang et al, 2001, 2002). Additionally, Chen et al (2011) made a specific introduction of *S. sinensis* in English as well as an attempt to differentiate it from *S. hominis*. Several of the mentioned studies have also provided molecular evidence of differentiating *S. sinensis*

from other *Sarcocystis* species.

The fourth *Sarcocystis* species mentioned by Dubey et al (2014) in cattle, *Sarcocystis* sp. was named as *S. sinensis* in the original publication. Suppression of the name *S. sinensis*, would, in our view, lead to further confusion in scientific literature. For these reasons, we consider that *S. sinensis*, as described in 1995 by Zuo et al, remains as a valid name. Probably, ICZN should provide a statement about this topic.

Huong & Uggla named a new species as *S. dubeyi* n. sp. (PROTOZOA: SARCOCYSTIDAE) in water buffalo (*Bubalus bubalis*) in 1999. Samples from esophagus, tongue, heart, masseter, cervical, scapular, diaphragm, psoas, thigh, and abdominal muscles were collected from 60 carcasses of adult beef water buffaloes from Vietnam. They reported 13% prevalence rate of *S. dubeyi* from examined animals and presented the morphological structures under LM and TEM. They did not report any attempts at experimental infection. Their abstract states "The definitive host of *S. dubeyi* was not determined, but it could possibly be humans or other primates". On what basis was this possibility suggested? Should experiments not have attempted to use cats and dogs as definitive hosts, given their frequent role as definitive hosts for species of *Sarcocystis* using domesticated animals as intermediate hosts? Later, Dubey et al (2014) stated the "*S. dubeyi* (definitive host unknown but not cat or dog)" but without citing published evidence for this parenthetical statement. According to the morphological structure under LM and TEM (Huong et al, 1999), *S. dubeyi* is similar to *S. sinensis*. Moreover, there are no reports of gene sequences of *S. dubeyi* reported on the GenBank. Based on the fact that *S. sinensis* was reported earlier, and based on the fact that more experimental infection attempts have been made in searching for its definitive host, we hold that *S. sinensis* deserves priority. If the two taxa are to be synonymized, we recommend that *S. dubeyi* be relegated as a junior synonym in accordance to the priority statement of the ICZN.

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