



# INVESTIGATION INTO THE COCCIDIA (APICOMPLEXA: EIMERIIDAE) WITHIN CAPTIVE SNAKES OF THE GENUS *BOIGA* (CAT SNAKES)

LARAMIE COUNTY  
COMMUNITY COLLEGE  
WYOMING

Jessica L. Marsh, James A. Erdmann, Dr. Ami L. Wangeline, and Dr. Zachary P. Roehrs  
Department of Biology, Laramie County Community College, Cheyenne, WY



## Abstract

Coccidia (Apicomplexa: Eimeriidae) are protozoan intestinal parasites that infect vertebrates around the world. However, little is known about the coccidia that infect reptiles and there is the potential of finding many species unknown to science. Currently, there are 33 recognized snake species in the genus *Boiga* (cat snakes; Colubridae: Colubrinae) and to date, only one species of coccidia (*Caryospora kalimantanensis*) has been described infecting a captive *Boiga dendrophila*. In this study, fecal samples collected from four captive species of *Boiga* (*B. cyanea*, *B. dendrophila*, *B. irregularis*, *B. nigriceps*), one *Bothriopsis bilineata*, one *Gonyosoma oxycephalum*, and one *Gyalopion canum* housed at the University of Northern Colorado, Greeley, Colorado were examined for potential infection with coccidia. After standard flotation, samples were examined using Nomarski interference-contrast optics light microscopy at 1,250x magnification. To date 10 of 19 *Boiga* individuals have been examined, with no oocysts found in these species. The lack of infection could be because this is a healthy captive population of snakes that are not infected with coccidia. However, coccidia have been found in other seemingly healthy captive populations and infections can be intermittent even in non-captive individuals so we cannot rule out the possibility of coccidia within this population until all samples have been examined.

## Introduction

The family Colubridae is one of the largest families of snakes containing approximately 70% of described snake species (Lawson et al. 2005). Colubrids are found on every continent excluding Antarctica, but have the greatest diversity in temperate and tropical regions (Duszynski and Upton 2010). Like most vertebrates, colubrids may become infected with coccidian parasites (Apicomplexa: Eimeriidae). Apicomplexa is a phylum of parasitic protists characterized by an apical complex present at some stage in their development (Duszynski and Upton 2010). Eimeriidae are intestinal parasites that have a direct life cycle infecting epithelial cells of the hosts' intestinal wall. Relatively little is known about the biodiversity of coccidia infecting snakes in general, and only 170 species of coccidians have been described from the nearly 3,400 species of snakes (Duszynski and Upton 2010). This lack of coccidia research is not unusual as many vertebrates have not been thoroughly examined for these parasites. However, the fact that many colubrids are venomous presents different challenges for the collection of fecal matter. We were able to obtain 117 fecal samples of 33 species of snakes from Dr. Stephen Mackessy's lab at the University of Northern Colorado who keeps many captive colubrids for his research on venom.

To better understand the interaction and importance of these parasites with their host species and in their communities, we must first document and describe species of coccidia present and their associated hosts. In this study, we have focused on a subset of fecal samples from snakes in the genus *Boiga*. There are 33 recognized snake species in the genus *Boiga* commonly referred to as the cat snakes (Colubridae: Colubrinae). The geographic distribution of these snakes runs from the northern coast of Australia up into southern China and west to parts of India. To date, two studies have examined two species of *Boiga* (*B. dendrophila*, *B. irregularis*; Fig. 1A and B) and noted the presences of *Caryospora* spp., *Isospora* spp. and *Sarcocystis* spp., but did not identify these coccidia to species, and only one species of coccidia (*Caryospora kalimantanensis*) has been described infecting a captive *Boiga dendrophila* (Fig. 2; Caudell et al. 2002; Modry and Koudela 1997). In this study, we examine fecal samples of four species of *Boiga*: *Boiga cyanea* (green-headed catsnake), *Boiga dendrophila* (mangrove catsnake), *Boiga irregularis* (brown tree snake), and *Boiga nigriceps* (black-headed cat snake; Fig 1A–D). We also examined fecal samples from *Bothriopsis bilineata* (Amazonian palm viper), *Gonyosoma oxycephalum* (red-tailed ratsnake), and *Gyalopion canum* (western hooknose snake) which also have not been previously examined for coccidia (Fig. 1E–G; Duszynski and Upton 2010).

## Methods

Fecal samples were collected from snakes in Dr. Stephen Mackessy's lab at the University of Northern Colorado (Greeley, Colorado). Samples tubes were individually numbered and samples were stored in a solution of potassium dichromate ( $K_2Cr_2O_7$ ) until they could be examined. For each sample, 8–10 drops of fecal material in  $K_2Cr_2O_7$  was added to a 15 mL tube and filled with Modified Sheather's Sugar Solution (1.9 M) for standard flotation (Sheather 1923). Oocysts were allowed to float onto a clean cover slip for 5–10 minutes and then transferred to a clean microscope slide. Slides were examined with an Olympus BX-40 light microscope at 1,250x magnification and using Nomarski interference-contrast optics. Pictures of oocysts were taken with a DP-20 digital camera mounted to the microscope. Pictures were examined and measurements were taken using cellSens Standard 1.8.1 software (Olympus, Center Valley, Pennsylvania).

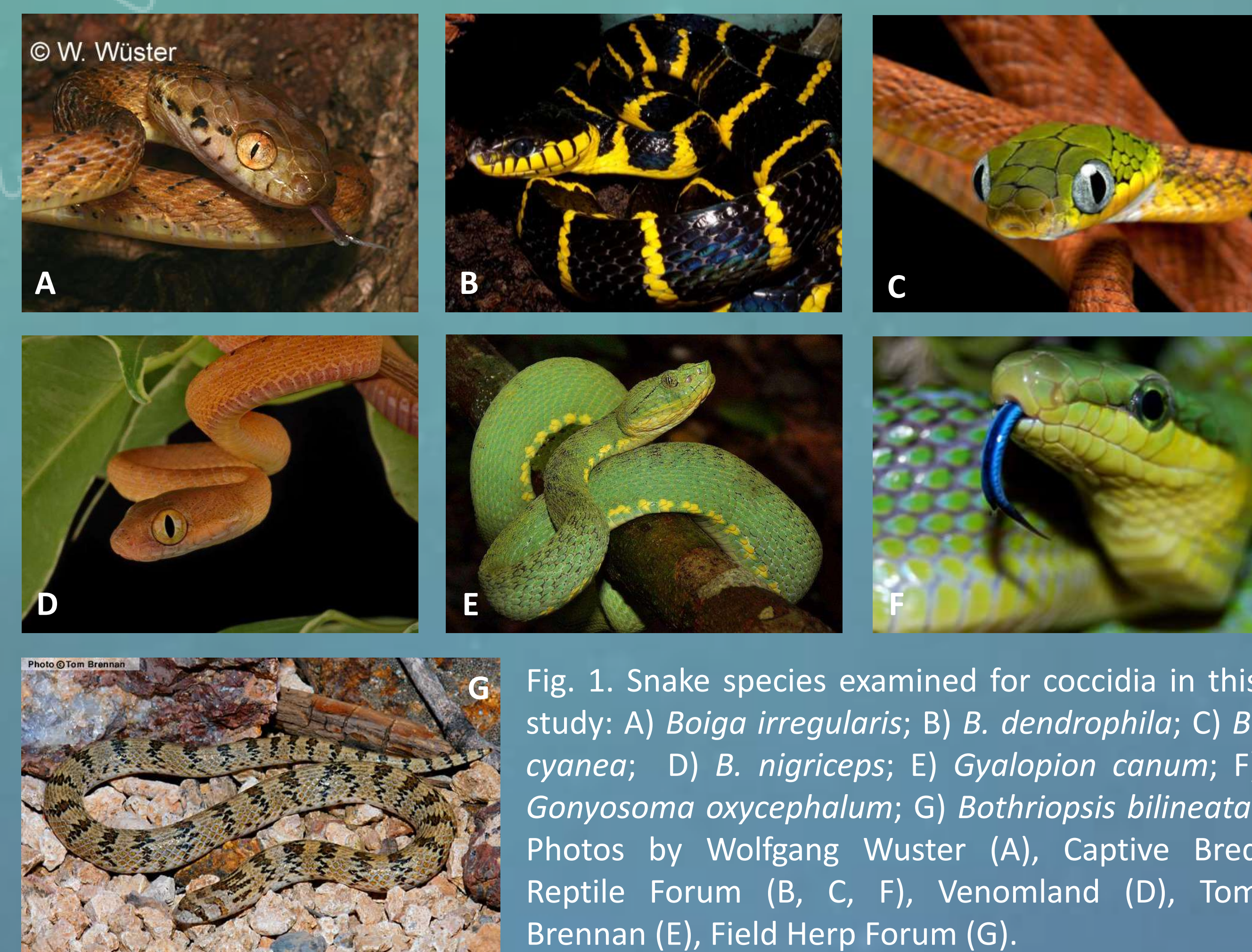


Fig. 1. Snake species examined for coccidia in this study: A) *Boiga irregularis*; B) *B. dendrophila*; C) *B. cyanea*; D) *B. nigriceps*; E) *Gyalopion canum*; F) *Gonyosoma oxycephalum*; G) *Bothriopsis bilineata*. Photos by Wolfgang Wuster (A), Captive Bred Reptile Forum (B, C, F), Venomland (D), Tom Brennan (E), Field Herp Forum (G).

## Results and Discussion

To date we have examined fecal samples from one *B. cyanea*, two *B. dendrophila*, five *B. irregularis*, two *B. nigriceps*, one *Bt. bilineata*, one *G. oxycephalum*, and one *G. canum* and have not observed oocysts in these 13 samples (15 slides). The lack of oocysts observed could be the result of multiple different factors. First, the lack of infection could result from the fact that these samples came from a healthy captive population of snakes that are not infected with coccidia. This could be because these snakes came into captivity uninfected or were not re-infected in captivity after an original infection ran its course. From the stand point of the health of this captive population, this would be a positive result. However, coccidia have been found in other captive populations, including private collections and zoos. Otherwise healthy looking and behaving snakes have been found shedding oocysts thus, despite the generally clean conditions required in captive facilities (as mandated by IACUC protocols), captivity alone does not negate the chances of infection (Modry and Koudela 1997; Papini et al. 2011; Pasmans et al. 2008; Upton et al. 1989). Second, coccidian infections are often intermittent within wild and captive populations and individuals may only show signs of being infected when oocysts are shed in the feces (Pasmans et al. 2008). The samples that were collected may be from snakes that were not infected or samples may have been collected during the prepatent period or an intermittent stage where despite being infected, no oocysts were being shed.

It cannot be stated definitively that this population is free of coccidia until all samples have been examined. Currently 14 samples from *Boiga* remain to be examined, as well as 90 fecal samples from 26 other species of snakes from this same captive population. Future efforts will focus on examining the rest of the samples and if infected samples are detected, the species of coccidia will be identified and/or new species named. In the event that an animal is found to be infected the MacKessy Lab will be contacted so that the captive individuals can be treated. It is only based on identification of coccidia present in many host species and individuals that we can begin to build a picture of coccidia biodiversity and host specificity to determine if these coccidia are a health risk and potentially pose a risk to other captive individuals (Duszynski and Upton 2010).

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## Acknowledgements

We thank Laramie County Community College for the use of their labs and equipment. This project would not have been possible without Dr. Stephen Mackessy and his students at the University of Northern Colorado (Greeley, Colorado) for the collection and donation of the fecal samples. This research was supported by NIH Grant # P20 RR016474 from the INBRE Program of the National Center for Research Resources. Its contents are solely the responsibility of the author and do not necessarily represent the official views of NIH.

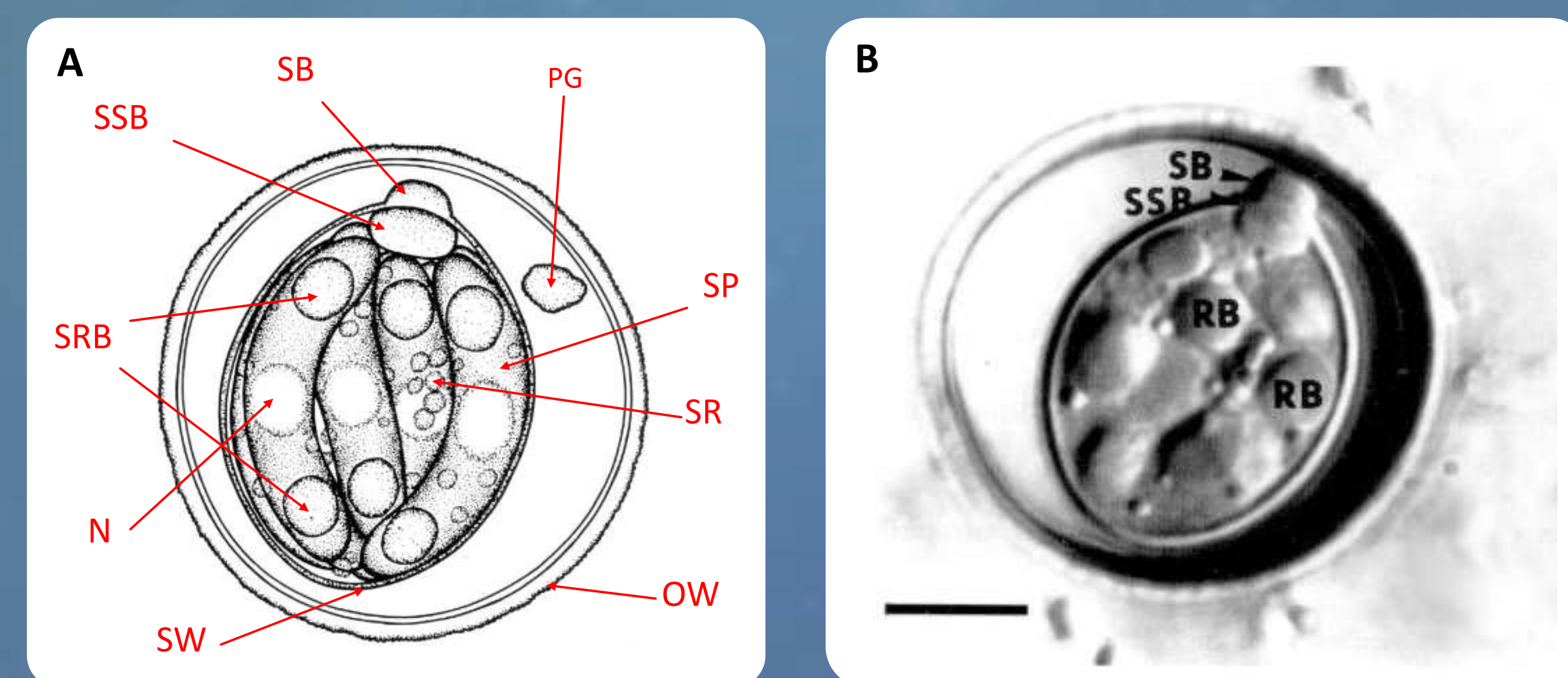


Fig. 2. Modified from Mordy and Koudela (1997). A) Composite line drawing of *Caryospora kalimantansis*; OW: oocyst wall, SW: sporocyst wall, N: nucleus, SRB: sporocyst refractile body, SSB: substieda body, SB: stieda body, PG: polar granule, SP: sporozoite, SR: sporocyst residuum. B) Type picture of a *C. kalimantansis* oocysts; SB: stieda body, SSB: substieda body, and RB: sporocyst refractile body.