NOTE
Wildlife Science

Phylogenetic characterization of *Isospora jaracimrmani* oocysts from a veiled chameleon (family Chamaeleonidae; *Chamaeleo calyptratus*) reared at a zoo in Ishikawa, Japan

Fitrine EKAWASTI1,2)#, Kazuya KITAGAWA3)#, Hiroshi DOMAE3)#, April Hari WARDHANA1,2,4)#, Junki NAGASAWA2)#, Tomoyuki SHIBAHARA2,5)#, Masaharu TOKORO6)#, Kazumi SASAI2,7)# and Makoto MATSUBAYASHI2,4,7)*#

1)Indonesian Research Center for Veterinary Science, Bogor 16114, Indonesia
2)Department of Veterinary Science, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka 598-8531, Japan
3)Ishikawa Zoo, Nomi, Ishikawa 923-1222, Japan
4)Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Airlangga University, Surabaya 60115, Indonesia
5)Division of Pathology and Pathophysiology, National Institute of Animal Health, NARO, Tsukuba, Ibaraki 305-0856, Japan
6)Department of Parasitology, Graduate School of Medical Sciences, Kanazawa University, Kanazawa 920-8640, Japan
7)Asian Health Science Research Institute, Osaka Prefecture University, Osaka 598-8531, Japan

ABSTRACT. Oocysts of *Isospora* sp. were detected in the feces of a veiled chameleon (family Chamaeleonidae; *Chamaeleo calyptratus*) kept at a zoo in Ishikawa, Japan. Phylogenetic analysis placed the sequence in the cluster of *Isospora* spp. isolated from reptiles. Based on a comparison of morphological data of ten previously reported *Isospora* species from the Chamaeleonidae family, this isolate was morphologically similar to *I. jaracimrmani*, which has been considered to be a virulent species. This case study suggests the possibility that species of *Isospora* might not always cause disease because the animal that shed these oocysts showed no symptoms for more than two months.

KEY WORDS: *Isospora*, Japan, oocyst, veiled chameleon

The veiled chameleon (family Chamaeleonidae; *Chamaeleo calyptratus*) is endemic to the southwestern area of the Arabian Peninsula and is one of the most popular chameleon species in the world. They prefer humid coastal lowlands, coastal slopes, and high plateaus and generally feed on insects such as locusts, grasshoppers, and crickets, by capturing them with their sticky tongues. Chameleons sometimes consume the leaves of plants as a source of water, especially during the dry season [9].

To date, ten species of protozoan coccidian parasites, *Isospora*, have been isolated and described from seven members of the Chamaeleonidae from four geographic areas, Africa, the Republic of Madagascar, the Seychelles, and the Republic of Yemen (summarized by McAllister) [7] (see Table 1). Among them, *I. jaracimrmani* has been reported to cause serious health problems such as weight loss and weakness in infected hosts [11, 12]. In this study, isosporan oocysts were isolated from a veiled chameleon reared at a zoo in Japan. We compared the morphology of the isolates with that of previously reported isolates and analyzed the genetics to determine the species and phylogenetic position.

A veiled chameleon (1-year-old) kept in captivity at a zoo in Ishikawa Prefecture, Japan, since its birth in September 2017, was periodically screened for parasites as a routine examination before exhibition based on examination of feces by the sucrose centrifugal flotation method [19]. The chameleon did not show any clinical symptoms when fecal samples were collected. Oocysts of *Isospora* sp. were detected on November 8, 2018, and January 27, 2019 (Fig. 1), and an anti-coccidiostat, 5 mg of toltrazuril (0.1 ml) (Bayer
The database revealed a nucleotide identity of 99.9% with differences were observed in the sequences among these samples (Accession No. LC617200). BLAST searches of the GenBank J. Vet. Med. Sci. 83(8): previous detected in Chamaeleonidae, as summarized in Table 1, this isolate was most similar to an isolate of Isospora sp. previously reported primer pairs targeting the 18S rRNA gene [6]. Phylogenetic trees were constructed as described previously [4]. Briefly, sequences were aligned using Clustal X (Version 2.0) [5], and all gaps were deleted. Maximum likelihood analyses with 500 bootstrap replicates were performed using the MEGA software package (version 10.0) [18], and a phylogenetic tree was constructed using the substitution model with optional parameters of the Tamura-Nei model with (G+I) distribution [17].

**Table 1.** Comparison of morphology of *Isospora* spp. in the present study and in the ones isolated from the Chamaeleonidae

| Species          | Mean length × width (range) (µm) | Mean L/W (range) | Mi | OR | PG | Mean length × width (range) (µm) | Mean L/W (range) | SB | SSB | SR | Host          | Locality | Refe
|------------------|----------------------------------|------------------|----|----|----|----------------------------------|------------------|----|-----|----|----------------|-----------|-------|
| **This study**   | 35.5 (28.2–42.4) × 23.4 (19.4–27.4) | 1.5 (1.14–1.99) | -  | -  | -  | 14.0 (12.6–16.1) × 11.3 (10.0–13.0) | 1.27 (1.11–1.56) | +  | +   | +  | Chamaeleo calyptratus | Japan     |
| *Isospora brygooi* | 20.7 (17–25) × 19.3 (16–23) | 1.1              | -  | -  | +  | 12.2 (12–13) × 8.1 (8–9) | 1.5              | +  | +   | +  | Furetiera pardalis | Namibia   |
| *Isospora freedi* | 23.7 (21–26) × 21.2 (18–24) | 1.1              | -  | -  | +  | 13.9 (13–14) × 10.3 (9–11) | 1.34             | +  | +   | +  | Chamaeleo calyptratus | Namibia   |
| *Isospora jaracimrmani* | 38.4 (35.2–42.8) × 25.6 (23.8–27.0) | 1.5             | -  | -  | -  | 15.9 (14.8–17.0) × 11.2 (10.4–12.0) | 1.4             | +  | -   | +  | Chamaeleo calyptratus | Yemen     |
| *Isospora mandalai* | 36.9 (34–39) × 31.0 (26–35) | 1.2              | -  | -  | -  | 15.3 (14–16) × 11.1 (10–12) | 1.37             | +  | +   | +  | Chamaeleo dilepis | Namibia   |
| *Isospora mesnili* | 30 (diam) | -  | -  | -  | -  | 16 × 10 | 1.6 | -  | -   | +  | Chamaeleo chameleon | Algeria   |
| *Isospora muriya* | 23.6 (21.5–25) × 21.9 (21–23) | 1.08             | -  | -  | -  | 12.4 (12–13) × 8.7 (8–10) | 1.4              | +  | +   | +  | Triceros jacksoni | Kenya     |
| *Isospora necassi* | 26.3 (21–30) × 24.0 (20–27) | 1.1              | -  | -  | -  | 12.8 (12–14) × 9.8 (8–10) | 1.31             | +  | +   | +  | Triceros melleri | Tanzania  |
| *Isospora taizii* | 28 × 22 | 1.3 | -  | -  | -  | 13 × 9 | 1.4 | -  | -   | +  | Chamaeleo calyptratus | Yemen     |
| *Isospora tigris* | 22.5 (19–24) × 18 (16–20) | 1.25             | -  | -  | -  | 13.6 (12–15) × 7.6 (6–8) | 1.9              | +  | +   | +  | Caluma tigris | Republic of the Seychelles |
| *Isospora wildi* | 25 (22–28) × 21 (18–24) | 1.17             | -  | -  | -  | 12.3 (12–13) × 9.7 (9–10) | 1.28             | +  | +   | +  | Calima dilepis | Tanzania  |

*Blank: data not available, Mi: microple, OR: oocyst residuum, PG: polar granules, SB: stieda body, SSB: sub-stieda body, SR: sporocyst residuum.*

Figure 1. *Isospora* oocysts detected in the feces of a veiled chameleon.
sexlineatus (family Lacertidae) and 99.8% from I. abdallahi (Accession No. KU180240) isolated from Acanthodactylus boskianus (family Lacertidae). We then constructed a phylogenetic tree using the 18S rRNA gene sequence obtained in the present study and published the sequences of related parasites. The sequence obtained in the present study was placed in a clade with the closely related Isospora spp. from reptiles (Fig. 2).

Isospora spp. that infect lizards are thought to show a high degree of host specificity [3], and more than 100 species of Isospora spp. have been described from reptiles, mainly based on the morphological data of oocysts and the host animal species [8]. The isolate in the present study was morphologically similar to I. jaracimrmani, which has previously been suggested to show pathogenicity [11, 12]. Although oocysts were not collected from the previously treated chameleon, both the previous case and the veiled chameleon in the present study did not show severe symptoms before administration of coccidiostats. Although these animals may have been lightly infected, this species of Isospora might not always cause the disease. One of the possible transmission routes might be breeding environments, including soils contaminated with oocysts. Although no sequence data of Isospora spp. from members of the Chamaeleonidae are available, the sequence from the isolate was placed in the cluster of Isospora spp. from reptiles of other families. However, compared to data available for other species of Coccidia (e.g., Eimeria spp.), the sequence data of Isospora spp. are largely lacking and, thus, there is a necessity of molecular analysis of the isolates and of other gene loci for understanding the classification or identification of parasites and for further evaluation of pathogenicity.
POTENTIAL CONFLICTS OF INTEREST. The authors declare that they have no conflicts of interest.

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