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Morphological description of *Isospora alyousifi* nom. n. for *I. acanthodactyli* Alyousif et Al-Shawa, 1997 (Apicomplexa: Eimeriidae) infecting *Acanthodactylus schmidti* (Sauria: Lacertidae) in Saudi Arabia

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Abstract: To date, three species of *Isospora* Schneider, 1881 have been described from lizards of the genus *Acanthodactylus* Wiegmann. Two of these, although representing separate species parasitizing two different hosts, *Acanthodactylus boskianus* Daudin in Egypt and *A. schmidti* Haas in Saudi Arabia, were described under the name *Isospora acanthodactyli*. The third species is *Isospora abdallahi* Modrý, Koudela et Šlapeta, 1998 from *A. boskianus* in Egypt. In the present study, *Isospora alyousifi* nom. n. is proposed to accommodate *Isospora acanthodactyli* Alyousif et Al-Shawa, 1997 (homonym of *I. acanthodactyli* Sakran, Fayed, El-Toukhy et Abdel-Gawad, 1994) and its redescription based on newly collected material is provided.

Keywords: homonymy, redescription, oocysts, endogenous stages, coccidia, sandy fringed-toed lizard, Riyadh

Lacertid lizards of the genus *Acanthodactylus* Wiegmann are widely distributed from West Africa to the Middle East (Harris and Arnold 2000, Harris et al. 2003, Rifai et al. 2003). Apicomplexan parasites of the genus *Isospora* Schneider, 1881 were first reported from lacertid lizards of the genus *Acanthodactylus* by Sakran et al. (1994), who described *Isospora acanthodactyli* from *Acanthodactylus boskianus* Daudin in Egypt. A few years later, Alyousif and Al-Shawa (1997), apparently unaware of the publication of Sakran et al. (1994), proposed *I. acanthodactyli* as a new species from *A. schmidti* from Saudi Arabia. The latter species thus became a homonym of *I. acanthodactyli* Sakran, Fayed, El-Toukhy et Abdel-Gawad, 1994. Meanwhile, Modrý et al. (1998) described another species, named by them as *I. abdallahi*, from *A. boskianus* from Egypt. Herein, we propose *Isospora alyousifi* nom. n. for *Isospora acanthodactyli* Alyousif et Al-Shawa, 1997.

MATERIALS AND METHODS

During a survey of coccidian parasites of lizards in the central region of Saudi Arabia, 40 adult sandy fringed-toed lizards, *Acanthodactylus schmidti* Haas, were collected by hand in the Thomama area (24°41'N, 46°42'E) in Riyadh City during November 2011. In the laboratory, the lizards were housed individually in small plastic cages. Individual faecal samples were collected daily from the ground of the cages and subjected to routine faecal examination using Sheather's sugar floatation method (Levine 1973). Faecal materials of infected lizards were then suspended in 2.5% potassium dichromate solution, incubated at 25 ± 1 °C and observed periodically to establish the sporulation time. Oocysts, sporocysts, sporozoites and other structures were measured and photographed using a calibrated ocular micrometer on an Olympus BX51 microscope with an Olympus DP71 camera. A total of 50 sporulated oocysts were measured. The measurements of the oocysts and the endogenous stages (in micrometres) are provided as arithmetic mean ± standard deviation, with range in parentheses, together with the shape index (ratio of length/width). For histological examination, parts of the infected intestine were fixed in 10% neutral buffered formalin and embedded in paraffin. Serial sections were stained with haematoxylin and eosin.

RESULTS

Of the 40 adult sandy fringed-toed lizards *Acanthodactylus schmidti* examined in this study, eight shed oocysts in their faeces. Initially, the examined faeces contained non-sporulated oocysts, oocysts in the early stages of sporulation and a few fully sporulated oocysts. The majority of recovered oocysts became fully sporulated within 12 hours at 25 ± 1°C when placed in 2.5% (w/v) aqueous potassium dichromate solution. The sporulated oocysts from all the examined lizards were found to belong to the genus *Isospora* and appeared to be of the same species. Below we present a morphological description of the oocysts and endogenous stages of this coccidian, which is considered conspecific with *Isospora acanthodactyli* Alyousif et Al-Shawa, 1997, which is a homonym of *I. acanthodactyli* Sakran, Fayed, El-Toukhy et Abdel-Gawad, 1994. To avoid this homonymy, a new name, *I. alyousifi*, is proposed and the species is redescribed on the basis of new material.

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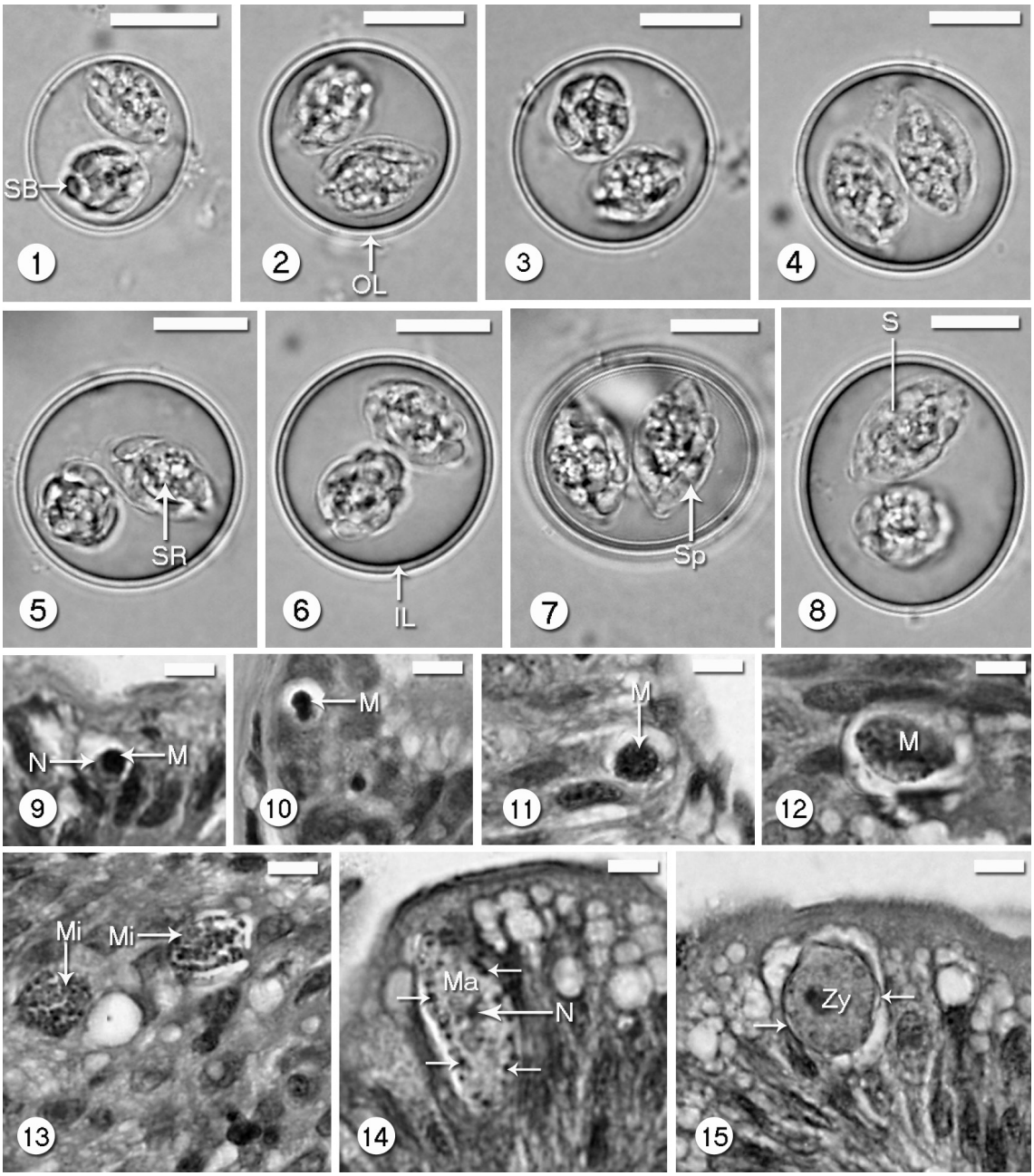
Isospora alyousifi nom. n. Figs. 1–16

Oocysts. Oocysts spherical to subspherical, 24.5 ± 2.5 (17–29) × 21 ± 2.5 (16–26), with length/width ratio of 1.11 (1.06–1.2) (Figs. 1–8, 16). Oocyst wall yellow, smooth, bilayered, 1.5 (1.2–1.6) thick, composed of thicker outer layer (about two thirds of total thickness – Fig. 2) and smooth inner layer (about one third of total thickness – Fig. 6). Micropyle, oocyst residuum and polar granules absent. Sporocysts ovoidal, 13.5 ± 1.5 (8–16) × 9 ± 1.5 (6–11), with smooth, single-layered wall, 0.5 thick; length/width ratio of 1.4 (1.1–1.7) (Fig. 8). Stieda body (Fig. 1) as well as sporocyst residuum present; residuum composed of numerous granules of nearly same size (Fig. 5). Sporozoites elongate with spherical anterior and posterior refractile bodies (Fig. 7).



Figs. 1–15. *Isospora alyousifi* nom. n. from the intestine of *Acanthodactylus schmidti*. **1–8.** Mature oocysts showing the wide size range and steady increase in size from the smaller to the larger oocysts. The oocyst surrounded with the outer layer (OL) and inner layer (IL) membrane, containing two sporocysts (S), each with Stieda body (SB), sporocyst residuum (SR) and four sporozoites (Sp). **9–15.** Endogenous development and endogenous stages. **9**, **10.** Early meronts (M) and nucleus shifted to one pole of enterocytes (N). **11.** Developing meront (M). **12.** Mature meront (M). **13.** Microgamonts (Mi) with a large number of small nuclei. **14.** Macrogamonts (Ma) with wall-forming bodies arranged at the periphery (arrows) and a centrally located nucleus (N). **15.** Zygote (young oocyst) (Zy) surrounded by thin envelope (arrows). Scale bars: 1–8 = 10 µm, 9–15 = 5 µm.

Endogenous stages. Observed within nuclei of enterocytes in posterior segment of small intestine. In heavily infected lizards endogenous stages also found in anterior intestine. Nuclei shifted to one pole in infected cells (Figs. 9, 10). As development of endogenous stages progressed, nuclei became gradually consumed and transformed into thin envelope around parasite (Fig. 15). Early trophozoites spherical (Figs. 9, 10), 3–5 in diameter. Developing meronts also spherical, 6–8 in diameter (Fig. 11). Mature meronts subspherical to ovoid, 11–13 × 6–8 (Fig. 12). Microgamonts subspherical, 7–9 × 5–7, distinguished by large number of small nuclei (Fig. 13). Macrogamonts ovoid to elliptical, 18–20 × 6–9, identified by wall-forming bodies arranged at periphery and by centrally located nucleus (Fig. 14). Zygotes (young oocysts) spherical, 12–14 in diameter (Fig. 15).

Type host: *Acanthodactylus schmidti* Haas.

New material deposited: Photomicrographs and slide with histological sections are deposited at the Zoology Department Museum, College of Science, King Saud University, Riyadh, Saudi Arabia (Coll. No. I/30/2012).

Sporulation: Exogenous and majority of recovered oocysts became fully sporulated within 12 hours at (25 ± 1°C).

Site of infection: Endogenous stages developed within the nuclei of the enterocytes.

Prevalence: 20% (8/40) in the Thomama area (24°41'N, 46°42'E) in Riyadh City, Saudi Arabia.

Etymology: The specific epithet is given in honour of Mohamed Alyousif, who first described the species.

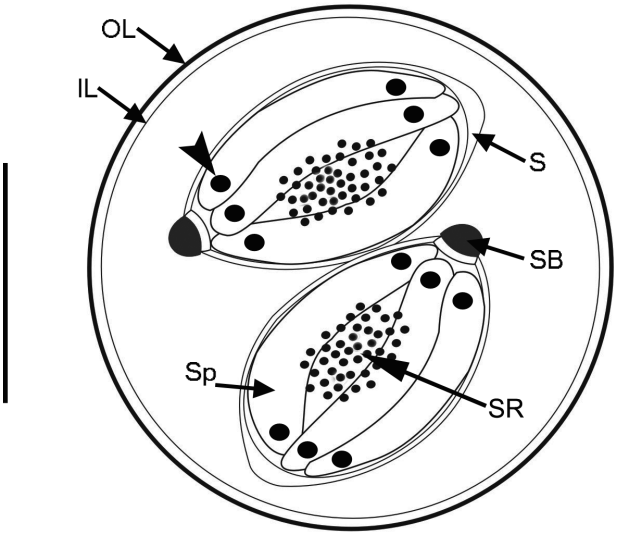


Fig. 16. Schematic drawing of an oocyst of *Isospora alyousifi* nom. n. The oocyst surrounded by the outer layer (OL) and inner layer (IL) membrane, containing two sporocysts (S), each one with Stieda body (SB), sporocyst residuum (SR) and four sporozoites (Sp); each sporozoite has two refractile bodies (arrowheads). Scale bar = 10 μm.

DISCUSSION

The oocysts of all members of the genus *Isospora* possess two sporocysts with four sporozoites in each and a single polar Stieda body (Barta et al. 2005). Descriptions of new isosporan species are traditionally based solely on oocyst morphology (e.g., Pellerdy 1974, Upton et al. 2001). In the 1990's, photomicrographic documentation became common (Duszynski 1999). However, this oocyst-based classification has proven problematic. The risk of identifying some eimerian coccidians solely by the morphology of their oocysts and sporocysts have been discussed by numerous authors (see Finkelman and Paperna 1994), and species of *Isospora sensu lato* are no exception (Lainson and Paperna 1999). As Berto et al. (2011) have recently argued, reliable classification is dependent on a statistically based evaluation of the morphometrics of the oocysts and, where possible, all the developmental stages of the species life cycle.

It is not uncommon that a species of parasite has been described from just a single infected host (Perkins et al. 2011). In terms of morphology, this small sample size may not permit an adequate examination of the variability present within a species. Thus, if the parasite is encountered again, slight morphological differences may drive investigators to describe it as a new species (Perkins et al. 2011). One set of issues in species identification, therefore, is an over-reliance of oocyst morphology for identification purposes, combined with inadequate sample sizes.

Three species of *Isospora* have been described from lizards of the genus *Acanthodactylus*. These species are *I. abdallahi* Modrý, Koudela et Šlapeta, 1998, *I. acanthodactyli* Sakran, Fayed, El-Toukhy et Abdel-Gawad, 1994

Table 1. Comparative descriptive measurements (in μm) of *Isospora alyousifi* nom. n. with morphologically similar species.

Species/ Reference	Host	Oocyst size	Oocyst shape and SI*	Sporocyst size	Sporocyst shape and SI*
Isospora abdallahi Modrý, Koudela et Šlapeta, 1998	*Acanthodactylus boskianus*	25.8 (24.5–29.0) × 23.9 (23.0–25.5)	Spherical or subspherical 1.07 (1.00–1.16)	15.4 (14–16) × 9.4 (9–10)	Ovoid 1.60 (1.50–1.80)
Isospora acanthodactyli Sakran, Fayed, El-Toukhy et Abdel-Gawad, 1994	*Acanthodactylus boskianus*	17.2 (16.4–18.8) × 16.4 (15.0–17.2)	Spherical 1.02	9.3 (7.4–10.4) × 5.9 (5.0–6.3)	Ovoid –
Isospora acanthodactyli Alyousif et Al-Shawa, 1997 (= *I. alyousifi*)	*Acanthodactylus schmidti*	27.9 (25.1–29.0) × 25.5 (22.7–27.8)	Spherical 1.09 (1.03–1.30)	11.6 (11.2–12.6) × 8.0 (7.5–8.4)	Ovoid 1.32 (1.27–1.54)
Isospora alyousifi nom. n. (present study)	*Acanthodactylus schmidti*	24.6 (17.0–29.0) × 21.0 (16.0–26.0)	Spherical 1.11 (1.06–1.20)	13.4 (8–16) × 8.5 (6–10)	Ovoid 1.40 (1.10–1.70)

*SI: shape index (length/width ratio)

and *I. alyousifi* nom. n. (syn. *I. acanthodactyli* Alyousif et Al-Shawa, 1997) (Table 1). *Isospora abdallahi* differs in having a thicker oocyst wall and the ranges of measurements of oocysts and sporocysts never overlap those of *I. alyousifi*. Although *I. acanthodactyli* looks similar to *I. alyousifi*, its oocyst wall is 2.2–3.8 µm thick compared to 1.5 µm only in *I. alyousifi* and the length of their oocysts and sporocysts does not overlap.

Alyousif and Al-Shawa (1997) overlooked the original description of *I. acanthodactyli* by Sakran et al. (1994) and used the same name for an apparently distinct species, the name of which thus became unavailable as homonym of the former one. A new name, *I. alyousifi*, is proposed and the species is redescribed based on new material from the same host, with similar size of oocysts and sporocysts, and identical other features as those of *I. acanthodactyli* Alyousif et Al-Shawa, 1997.

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