

Two new species of *Isospora* (Apicomplexa: Eimeriidae) from geckoes of the genus *Rhacodactylus* (Sauria: Gekkonidae) endemic to New Caledonia

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Abstract. Coprological examination of New Caledonian geckoes of the genus *Rhacodactylus* Fitzinger, 1843 revealed two new species of coccidia. *Isospora leachiana* sp. n. from *R. leachianus* (Cuvier, 1829) has oval, colourless oocysts, measuring 21–26 × 16–18.5 µm. Sporocysts are ellipsoidal, 11–12.5 × 6.5–8 µm, with distinct Stieda and substieda bodies. Oocysts of *Isospora sykorai* sp. n. from *R. ciliatus* (Guichenot, 1866) are elongately oval to cylindrical, 20–23.5 × 11–14 µm; sporocysts of this species are ellipsoidal, 10–11.5 × 7–8 µm, with a slightly pointed end and Stieda and substieda bodies. Infected geckoes did not exhibit any alteration of their health status.

The fauna of terrestrial vertebrates of New Caledonia is exceptional because of high level of endemism (over 86% in reptiles) (Chazeau 1993, Bauer and Sadlier 2000). Among the gekkonid hosts (Gekkota: Gekkonidae), the members of the Diplodactylinae represent a unique, diversified assemblage of geckoes inhabiting mostly Australia, New Caledonia and New Zealand. The New Caledonian genus *Rhacodactylus* Fitzinger, 1843 is probably the most peculiar as it contains the largest extant gecko *R. leachianus* (Cuvier, 1829) (Bauer and Sadlier 2000). Despite the diversity and uniqueness of the New Caledonian fauna, no species of coccidia have been reported from terrestrial vertebrates of this archi-pelago. In the present paper, a description of two new species of *Isospora* Schneider, 1875 parasitizing gekkonid hosts is given as first result of a research focused on parasitic protozoans of New Caledonian herpeto-fauna.

MATERIALS AND METHODS

This study is based on examination of both captive and wild-living geckoes of the genus *Rhacodactylus*, namely *R. auriculatus* (Bavay, 1869), *R. ciliatus* (Guichenot, 1866) and *R. leachianus*. Geckoes examined in facilities of European herpetologists were kept individually in glass terraria and faecal samples were collected daily from cages. Faecal samples were placed into plastic vials with 2.5% (w/v) potassium dichromate ($K_2Cr_2O_7$), mixed thoroughly, and submitted for parasitological examination. Additionally, a limited number of faecal samples were collected from seven specimens of *R. auriculatus* during field study in New Caledonia in September

2002. All specimens collected in nature were placed for 12 h into plastic boxes until they had defecated. Faecal samples were then placed into plastic vials with 2.5% potassium dichromate, mixed thoroughly, and transported to the Czech Republic. All faecal samples were routinely examined microscopically after concentration by flotation with modified Sheather's sugar solution (s.g. 1.30). Coccidian oocysts were measured and photographed using Nomarski interference contrast optics (NIC). Measurements were made using a calibrated ocular micrometer and are reported in micrometres as the means, followed by the ranges in parentheses. Additionally, fresh samples containing unsporulated isosporan coccidia were allowed to sporulate in Petri dishes at room temperature (20–23°C) and examined daily to determine the stage of sporulation.

RESULTS

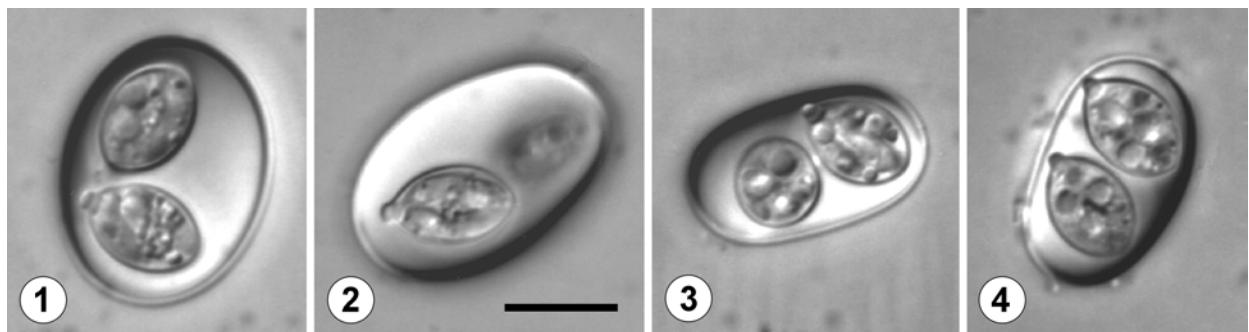
Coprological examination revealed presence of coccidian oocysts in 1 of 9 (11%) *R. leachianus* and in 1 of 8 (12.5%) *R. ciliatus* examined. No coccidia were found in faeces of *R. auriculatus*. Both recovered coccidian isolates evidently represent new species and are described as follows.

APICOMPLEXA: Eimeriidae

Isospora leachiana sp. n.

Figs. 1, 2, 5

Description. Oocysts oval, 23.4 (21–26) × 17.2 (16–18.5), SI 1.36 (1.24–1.53). Micropyle, oocyst residuum and polar granule absent. Oocyst wall smooth, colourless, bilayered, ca. 1 thick, outer layer significantly



Figs. 1–4. Nomarski interference contrast (NIC) micrographs of sporulated oocysts of *Isospora* spp. described from *Rhacodactylus* spp., all at the same scale. **Figs. 1, 2.** *Isospora leachiani* sp. n. **Figs. 3, 4.** *Isospora sykorai* sp. n. Scale bar = 10 µm.

thicker. Sporocysts ellipsoidal, 11.8 (11–12.5) × 7.1 (6.5–8), with smooth, colourless and unilayered sporocyst wall; sporocyst SI 1.67 (1.5–1.85). Distinct Stieda body present, dome-like, 1–1.5 high and 2 wide, substieda body subglobular, 2–2.5 in diameter. Sporozoites elongate, arranged head-to-tail within sporocyst. Each sporozoite with spherical to subspherical anterior and posterior refractile body. Sporocyst residuum present as granules of irregular size, <1 in diameter, scattered among sporozoites.

Type host: *Rhacodactylus leachianus* (Cuvier, 1829), the giant gecko (Sauria: Gekkonidae: Diplodactylinae).

Type locality: Based on the owner's information, the examined animal originated from the wild, being captured at Illot Brosse (22°43'S, 167°27'E, Province Sud, New Caledonia) three years prior the examination.

Site of infection: Unknown, oocysts recovered from faeces.

Prevalence: One (11%) of 9 examined captive *R. leachianus* was infected.

Sporulation: Exogenous. Oocysts recovered from preserved faeces contained a single spherical sporont. Sporulation was completed within 36 h at ~22°C.

Type material: Phototypes are deposited in Department of Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic (no. R 54/01).

Eymology: The specific epithet *leachiani* is the genitive from the host species name *leachianus*.

Isospora sykorai sp. n.

Figs. 3, 4, 6

Description. Oocysts elongately oval to broadly cylindrical, 21.1 (20–23.5) × 12.9 (11–14); SI 1.65 (1.43–2.0). Micropyle, oocyst residuum and polar granule absent. Oocyst wall smooth, colourless, bilayered, rather thin (<1), with inner layer being much thinner. Sporocysts ellipsoidal, 10.8 (10–11.5) × 7.2 (7–8); SI 1.5 (1.31–1.57), with slightly pointed end and with smooth, colourless and unilayered sporocyst wall. Stieda body present, dome-like, 1.5 high and 1.5 wide, substieda body ellipsoidal, 0.7–1 high and 1.5 wide. Sporozoites elongate, arranged head-to-tail within

sporocyst. Each sporozoite with spherical anterior and posterior body of unequal size and barely visible spherical nucleus situated centrally. Sporocyst residuum present as few, very small granules of equal size, <1 in diameter, scattered among sporozoites.

Type host: *Rhacodactylus ciliatus* (Guichenot, 1866), the crested giant gecko (Sauria: Gekkonidae: Diplodactylinae).

Type locality: Unknown, the species is described from a captive host.

Site of infection: Unknown, probably the intestine.

Prevalence: 1/8 (12.5%).

Sporulation: Exogenous, the oocysts recovered from fresh faeces were unsporulated. Sporulation at room temperature (20–23°C) within 3 days.

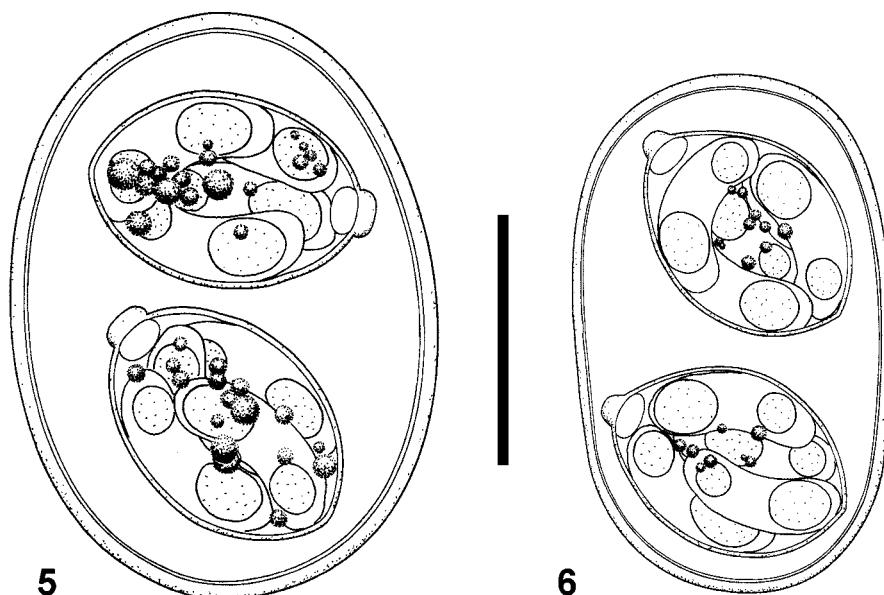
Type material: Phototypes are deposited in Department of Parasitology, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic (no. R 56/01).

Eymology: The species is named in honour of Zdeněk Sýkora (Merial, Czech Republic) in recognition in his generous support of our research.

DISCUSSION

New Caledonia is inhabited by more than 20 species of diplodactyline geckoes (Gekkonidae: Diplodactylinae) of three genera. The genus *Rhacodactylus* comprises 6 species. This group of saurians is typified by a high level of endemism, with many species being restricted to small geographic regions (Bauer and Sadlier 2000). Despite the small number of animals examined, two species of *Rhacodactylus* were found during this study to harbour coccidian infection. The diversity of eimerian coccidia parasitizing diplodactyline geckoes is probably comparable with coccidia of other saurian groups and further research might reveal a high number of other species.

Isosporoid coccidia of vertebrates are considered to be highly host specific. However, this assumption is mostly speculative in the case of species parasitizing reptiles, as studies aimed to trace the host specificity, and, in special, cross-transmissions experiments are lacking. Therefore, the coccidian species described here



Figs. 5, 6. Composite line drawings of sporulated oocysts. **Fig. 5.** *Isospora leachiani* sp. n. **Fig. 6.** *Isospora sykorai* sp. n. Scale bar = 10 μ m.

as new are further compared with all isosporans known from the Gekkota. Based on the recent systematic concept of gekkonid hosts, the diplodactyline geckoes are treated as a subfamily of Gekkonidae. Moreover, a monophyletic origin and distribution separates this group of saurians from other gekkonid hosts (Bauer 1990). Up to date, there are no species of coccidia described and named from hosts belonging to the Diplodactylinae. The only reference on isosporoid coccidia from these hosts is that of Cannon (1967) noting the presence of an unidentified *Isospora* in *Saltuarius* (originally *Phyllurus*) *cornutus* from Australia. So far, there are 24 named species of *Isospora* reported from members of the Gekkonidae (<http://biology001.unm.edu/~coccidia/home.html>, Finkelman and Paperna 2002). All the named species differ significantly by their shape index, as none have oocysts as elongate as those of *Isospora leachiani* (SI = 1.36) and *I. sykorai* (SI = 1.65). These differences justify the present descriptions as new species.

The *Rhacodactylus* specimens examined in this study did not show any sign of the alteration of health, regardless of high numbers of oocysts expelled in faeces and the long patency recorded. The pathology of coccidian

infections caused by *Isospora* spp. in captive reptiles is variable. Although there are reports on mortality and pathological findings (McAllister et al. 1995, Modrý and Koudela 1998, Kim et al. 2002), clinical isosporosis is probably a multifactorial disease, reflecting other con-current infections, as well as various environmental conditions.

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REFERENCES

BAUER A.M. 1990: Phylogenetic systematics and biogeography of the Carphodactylini (Reptilia: Gekkonidae). Bonner Zoologische Monographien, 30, 217 pp.

BAUER A.M., SADLIER R.A. 2000: The Herpetofauna of New Caledonia. Society for the Study of Amphibians and Reptiles, Ithaca, New York, 310 pp.

CANNON L.R.G. 1967: New coccidia from Australian lizards I. *Isospora*. Parasitology 57: 227–235.

CHAZEAU J. 1993: Research on New Caledonian terrestrial fauna: achievements and prospects. Biodiv. Lett. 1: 123–129.

FINKELMAN S., PAPERNA I. 2002: The endogenous development of four new species of *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) from Australian geckoes. Syst. Parasitol. 51: 59–71.

KIM D.Y., MITCHELL M.A., BAUER R.W., POSTON R., CHO D.Y. 2002: An outbreak of adenoviral infection in inland bearded dragons (*Pogona vitticeps*) coinfecte with dependovirus and coccidian protozoa (*Isospora* sp.). J. Vet. Diag. Invest. 14: 332–334.

McALLISTER C.T., UPTON S.J., JACOBSON E.R., KOPIT W. 1995: A description of *Isospora amphiboluri* (Apicomplexa, Eimeriidae) from the inland bearded dragon, *Pogona vitticeps* (Sauria, Agamidae). J. Parasitol. 81: 281–284.

MODRÝ D., KOUDLA B. 1998: Isosporan infections of *Chamaeleo calyptratus* represent growing problem for its breeding in captivity. Rept. Amphib. Mag. 54: 38–41.

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