NEW INSIGHTS INTO REPTILIAN COCCIDAN INFECTIONS FROM TWO SPECIES OF INVASIVE GECKOS, THE MEDITERRANEAN HOUSE GECKO, HEMIDACTYLUS TURCICUS AND THE TROPICAL HOUSE GECKO, H. MABOUIA FROM THE NEW WORLD

By
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NEW INSIGHTS INTO REPTILIAN COCCIDAN INFECTIONS FROM TWO SPECIES OF INVASIVE GECKOS, THE MEDITERRANEAN HOUSE GECKO, HEMIDACTYLUS TURCICUS AND THE TROPICAL HOUSE GECKO, H. MABOUIA FROM THE NEW WORLD

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Abstract: Classification of coccidia at the generic and family levels was traditionally based on the life cycle, and the number of sporocysts and sporozoites in the exogenous stages (oocysts). The genus *Eimeria* reported from all vertebrate classes was defined by tetrasporocystic, dizoic exogenous oocysts. However, recent molecular and developmental studies on eimerid coccidia, support multiple linages of coccidia with tetrasporocystic, dizoic oocysts. Two of these lineages, *Choleoeimeria* and *Acroeimeria*, found in reptiles contain a suture on the sporocyst and epicytoplasmic development in the gall bladder or intestine and are considered phylogenetically distant from the genus *Eimeria* which infect birds and mammals and contain stiedal bodies on the sporocyst. However, there are several species of *Eimeria*-like coccidia from reptiles, which excyst via the suture in the sporocyst wall but contain intracytoplasmic development. These taxa do not fit the definition of *Eimeria* nor the definition of the genera *Choleoeimeria* or *Acroeimeria*. However, no sequence data is available for these *Eimeria*-like coccidian. It is unclear if they represent a currently undescribed *Eimeria*-like genus or should be included in *Choleoeimeria* or *Acroeimeria*. To address this issue, I examined the coccidia of two species of introduced house geckos (*Hemidactylus turcicus* and *H. mabouia*) from North America, and documented their oocyst morphology, endogenous development and obtained partial 18s rRNA sequences. My phylogenetic analyses of all available 18s rRNA sequences of *Eimeria*-like coccidia species from New and Old-World lizard species indicated 3 clades, that differed in site of infection (gall bladder or intestine) and development (epicytoplasmic or intracytoplasmic). My analysis strongly suggests that a third *Eimeria*-like genus of coccidia infects lizard hosts. This work indicates that oocyst morphology is not useful in differentiating between these genera and obtaining oocyst morphology, endogenous development, and sequence data in future *Eimeria*-like species descriptions will be critical in our understanding of their taxonomical position and phylogenetic relationships. Additionally, my partial sequence of the 18s rRNA gene for *Isospora hemidactylus* obtained during this study and resulting phylogenetic analysis supports previous phylogenetic studies that *Isospora* species from lizards are not a monophyletic group, and suggests a paraphyletic origin of *Isospora* species infecting lizard hosts.
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CHAPTER I

INTRODUCTION

Reptiles, specifically geckos, are some of the most invasive species in the world, with the genus *Hemidactylus* being one of the most cosmopolitan (Capinha et al., 2017). The genus, *Hemidactylus* has over 165 described species (approximately 191) and ten of those have spread across continents. These invasive species of *Hemidactylus* dispersed across the world, through anthropogenic movements following the flow of commerce and human travel. Their dispersal and association with human activities has been occurring for decades, dating back as far as the Atlantic slave trade (Agarwal et al., 2021). During this time, several *Hemidactylus* gecko species were introduced and spread throughout the Neotropics from their native locations throughout Africa and Asia (Carranza and Arnold, 2006; Lajmi et al., 2016). Some of these species of *Hemidactylus* geckos were implicated to have spread along caravan routes in the Middle East. In North America, the introduction of multiple *Hemidactylus* species is more recent, starting a little over a century ago around 1910. Since their North American introduction, multiple species of *Hemidactylus*, including *Hemidactylus turcicus*, *Hemidactylus frenatus*, *Hemidactylus garnotii*, and *Hemidactylus mabouia* have been established throughout various regions of North America (Agarwal et al., 2021).
However, and within the genus, *Hemidactylus*, conserved morphological features and widespread distribution of various species, make it difficult to assign which lineage, species, and/or subspecies are present at a given location (Agarwal et al., 2021). Recent phylogenetic studies show that only some but not all lineages of *Hemidactylus* species are invasive. For example, molecular barcoding work on the tropical house gecko, *H. mabouia* which is native throughout Africa and invasive in the Neotropics and the United States, indicates that *H. mabouia* is a species complex consisting of at least twenty lineages/species (Agarwal et al., 2021). Morphological identification of tropical house geckos indicates there is one widely distributed species; whereas recent sequence data for *H. mabouia* is conflicting and indicates that *H. mabouia* is comprised of at least 20 species/lineages. However, the invasive populations of *H. mabouia* throughout the Neotropics and North America belong to only one of the twenty genetic lineages. Importantly, these recent findings on multiple cryptic species of *H. mabouia* and other *Hemidactylus* species make it difficult to understand the identification, distribution and host specificity of their pathogens and parasites (Gonzalez-Barrio et al., 2023) and particularly the coccida (Duszynski et al., 2000).

Although vertebrates in general and lizards specifically have been ignored for their coccidia diversity, of the sixteen extant families of lizards, the Gekkonidae have been examined more commonly for their coccidia infections compared to other families of lizards (Duszynski, 2021). In fact, no other family of lizards has been studied more thoroughly for coccidian parasites than geckos. Out of sixty-two genera in the family Gekkonidae, twenty-four genera and fifty-two species have been evaluated for coccidia parasites (Duszynski et al., 2000). As a result, Geckos in general, and more specifically
species of *Hemidactylus*, are known as hosts for multiple species of coccida (Paperna and Landsberg, 1989a, b). In fact, and compared to other geckos, species of *Hemidactylus* are the most surveyed geckos for coccidian parasites and represent 13% (7/52) of all gecko species examined for this group of intracellular parasites (Duszynski et al., 2000). However, although more thoroughly studied for their coccidian parasites than other gecko species, the difficulty of identifying *Hemidactylus* species based on morphology, makes this group of lizards more prone to difficulties in documenting the host specificity and host ranges of coccidian parasites that infect these hosts.

**Problems with the identification of coccidia parasites of lizards**

Similarly, to their hosts, and unlike other parasitic groups, coccidian parasites are also prone to difficulties in identification and assigning what host species they infect in nature and stemming from the difficulty of vouchering coccidian parasites and the lack of physical voucher specimens of coccidians deposited in museum collection for future DNA analysis. Traditionally, coccidia were predominantly described based on their oocyst morphology (Duszynski and Wilber, 1997). Morphological characteristics used to differentiate among coccidia species typically include oocyst length and width, number of sporocysts and sporozoites within an oocyst, the presence of polar granules, micropyle and micropyle features, among other characteristics (Duszynski and Wilber, 1997). However, it is difficult to preserve the oocyst morphology due to the degradation of sporulated oocysts over time and in typical fixatives and/or preservatives such as formaldehyde and 2-2.5% potassium dichromate (K2Cr2O7); the latter of which is used for oocyst to sporulate (Duszynski and Wilber, 1997).
As a result, Bandoni and Duszynski (1988) provided an alternative, by making a plea to use photomicrographs as an alternative to physical voucher specimens. Although this technique has become the gold standard for documenting coccidian morphology, it brings up an issue for DNA barcoding. Since there is no physical specimen voucher, there is no way to get sequences from studies that only deposited photographs. The recommendations of depositing photomicrographs for descriptions were established before sequence technology was common practice for parasitologists. Similarly, few previous studies have deposited physical vouchers of the host species for these coccidian species, making it difficult to differentiate what host species was infected with a specific coccidian species from locations where sympatric cryptic linages occur (McAllister et al. 1988). For example, Paperna and Landsberg, (1989a, b) reported multiple coccida species including *Acroeimeria lineri* from several gecko species including, *H. mabouia* collected from suburban dwellings near Pretoria, South Africa. However, recent molecular barcoding work by Agarwal et al. (2021) indicates that at least two lineages of *H. mabouia* occur in this region of South Africa, and since no host vouchers or biological coccidia material were deposited in a museum collection, it is unclear what lineage or linages of *H. mabouia* or coccidian parasites Paperna and Landsberg (1989b) were dealing with.

Similarly, to the difficulties in morphological identification of cryptic species of hosts among the *Hemidactylus* species, recent molecular barcoding data indicate problems with strictly using morphology for the oocysts of coccidians for generic and species level identifications (Megía-Palma et al. 2015). Classification of the coccidia at the generic and family levels was traditionally based on endogenous (intracellular)
development, their life cycles, and the number of sporocysts and sporozoites in the oocysts or exogenous stages (Levine, 1982; Jirku et al., 2002).

As with many intracellular parasites, the life cycles and development of coccidian parasites is complex. Some species are monoxenous, with one host in the life cycle, while other species require two or more hosts and are considered heteroxenous. The life cycle of a coccidian parasite involves three developmental phases including development and asexual reproduction such as merogony and sporogony, and sexual reproduction known as gamogony (Fig. 1). In a typical coccidian life cycle, the infective stage in this life cycle is a sporozoite and infects the host cell after being ingested accidentally by the host in the form of a cyst or by being inoculated through the bite of a hematophagous arthropod such as a tick or mosquito (Roberts et al., 2013). Once inside of the host cell the sporozoite develops to the trophozoite stage and multiplies through multiple fission known as merogony and an asexual form or reproduction to form multiple merozoites. These exist the host cell and enter other host cells to initiate further cycles of merogony. The number of merogony cycles is usually species specific but not well documented for most coccidia species and can vary from two to multiple cycles (Roberts et al., 2013). Once merogony is complete, the merozoites within epithelial host cells transform into gamonts and initiate sexual reproduction known as gametogony. Gamonts develop to male and female cells known as mico and macro gametocytes, respectively. The microgametocytes exit their host cells and enter host epithelial cells infected with macro gametocytes and form zygotes through fertilization. These zygotes known as oocysts usually exit their host epithelial cells and depending on the coccidian species are usually released in the hosts feces or urine into the external environment. Once in the external
environment multiple fission or sporogony, produces multiple sporozoite within the oocyst. These oocysts can survive in the external environment for a substantial amount of time until they are ingested by the appropriate host and the life cycle continues (Roberts et al., 2013).

In other variations of the life cycle and in some heteroxenous coccidia oocysts are released from the vertebrate definitive host and when ingested by a second vertebrate intermediate host, the sporozoite invade various tissues where asexual multiplication and multiple merogony cycles occur. After the appropriate number of merogony cycles, these merozoites remain dormant in the intermediate host tissues. Once an intermediate host is consumed by an appropriate vertebrate definitive host, the merozoites are digested out and infect appropriate epithelial cells within the definitive host. Merozoites within epithelial cells of the definitive host transform into gamonts and proceed through gametogony, eventually producing fertilized oocysts which are released from the definitive host into the external environment (Roberts et al., 2013). Finally, in other heteroxenous coccidia, and after merogony, gamogony occurs, the oocysts never exit the vertebrate definitive host, but instead sporulate in the tissue of their definitive vertebrate host. Once sporulated, the sporozoites exit the oocyst and migrate to the blood stream where they are ingested by arthropod or leech intermediate hosts. Within these invertebrate intermediate hosts, the sporozoites mature to the infective stage and are eventually injected into a vertebrate definitive host during blood feeding by the vector host, and where merogony, gamogony and sporogony occur (Barta et al., 2001).

Because of the variable life cycles of coccidians and their complex intracellular development within their hosts, coccidian descriptions have predominantly concentrated
on using the morphology of the oocyst to describe species and differentiate genera (Duszynski and Wilber, 1997). As a result, little information is available on the endogenous development for most of the described coccidian species. However, recent molecular and developmental studies strongly indicate that coccidian endogenous development, specific pathology, and site specificity maybe be critical in our understanding of coccidian phylogenetic relationships and species delimitation. For example, the genus Eimeria which has been traditionally differentiated by its tetrasporocystic, dizoic exogenous oocyst, is comprised of multiple linages which differ in their endogenous development, specific pathologies, and site specificity. As a result, and when combined with molecular data, recent but limited studies on the oocyst morphology, and documentation of endogenous development along with sequence and/or phylogenetic data suggest that tetrasporocystic, dizoic oocyst typically considered as Eimeria belong to multiple linages, that are not always their closest relatives (Jirku et al., 2002, 2009; Megía-Palma et al., 2015; Abdel-Baki et al., 2020). Similar results have been obtained for other coccidan genera, including Isospora, indicating that multiple genera share similar oocyst morphology (Megía-Palma et al., 2016)

In the following study, I evaluate coccidia infections in two species of introduced geckos (Hemidactylus turcicus and H. mabouia) collected in Oklahoma and Florida. Most of the work reported below deals with the Eimeria-like parasites with tetrasporocystic, dizoic exogenous oocysts that infect these two species of lizards; however, I also document an Isosporan coccidian, disporocystic, tetrazoic exogenous oocysts, that infected H. mabouia. Specifically, I document oocyst morphology and when
possible, the endogenous development and provide sequence data for coccidian species from four genera that infect these geckos.
Figure 1.1 Modified from Roberts et al. 2013. A typical *Eimerian* Life cycle. Merogony and sporogony (1 and 3) are stages of asexual reproduction. Gamogony (2) is a stage of sexual reproduction.
CHAPTER II

COCCIDIA OF LIZARD HOSTS

ABSTRACT: Classification of coccidia at the generic and family levels was traditionally based on the life cycle, and the number of sporocysts and sporozoites in the exogenous stages (oocysts). For example, the genus Eimeria reported from all vertebrate classes was defined by tetrasporocystic, dizoic exogenous oocysts. However, recent molecular and developmental studies on eimeriid coccidia, support multiple lineages of coccidia with tetrasporocystic, dizoic oocysts. Two of these lineages, Choleoeimeria and Acroeimeria, found in reptiles contain a suture on the sporocyst and epicytoplasmic development in the gall bladder or intestine, respectively; and are considered phylogenetically distant from the genus Eimeria which infect birds and mammals and contain stiedal bodies on the sporocyst. However, there are several species of Eimeria-like coccidia from reptiles, which excyst via the suture in the sporocyst wall but contain intracytoplasmic development. These taxa do not fit the definition of Eimeria nor the definition of the genera Choleoeimeria or Acroeimeria. However, no sequence data is available for these Eimeria-like coccidia and it is not clear if they represent a currently undescribed Eimeria-like genus or should be included in the Choleoeimeria or Acroeimeria. To address this issue, I examined the coccidia of two species of introduced house geckos.
(Hemidactylus turcicus and H. mabouia) from North America, and documented their oocyst morphology, endogenous development and obtained partial 18s rRNA sequences. My phylogenetic analyses of all available 18s rRNA sequences of Eimeria-like coccidia species from New and Old-World lizard species indicated 3 clades, that differed in site of infection (gall bladder or intestine) and development (epicytoplasmic or intracytoplasmic). My analysis strongly suggests that a third Eimeria-like genus of coccidia infects lizard hosts world-wide. This work indicates that oocyst morphology is not useful in differentiating between these genera and obtaining oocyst morphology, endogenous development, and sequence data in future Eimeria-like species descriptions will be critical in our understanding of their taxonomical position and phylogenetic relationships. Additionally, my partial sequence of the 18s rRNA gene for Isospora hemidactylus obtained during this study and resulting phylogenetic analysis supports previous phylogenetic studies that Isospora species from lizards are not a monophyletic group, and suggests a paraphyletic origin of Isospora species infecting lizard hosts.

INTRODUCTION

Protozoa known as coccidia (Apicomplexa: Conoidasida) are a diverse group of oocyst-forming, single-celled obligate intracellular parasites. As a group they infect most phyla of invertebrates and all classes of vertebrates. The disease they cause, coccidiosis, is recognized as the major health hazard in domestic animal husbandry, in zoo environments, and in wild animal populations (Roberts et al., 2013). Although the symptoms and pathology of coccidiosis vary greatly and depending on the host group and/or particular coccidian species, coccidia always develop within host cells and eventually destroy the epithelial cells they infect. As a result, some common symptoms
include diarrhea and host dehydration, but can also result in epithelium sloughing leading to host death (Roberts et al., 2013).

Classification of the coccidia at the generic and family levels was traditionally based on endogenous development, their life cycles, and the number of sporocysts and sporozoites in the oocysts or exogenous stages (Levine, 1982; Jyrki et al., 2002). Of the numerous genera of coccidia, the genus *Eimeria* (Eimeriidae) is one of the most common and species rich genera. The genus *Eimeria* has been reported from all vertebrate classes and originally defined by having tetrasporocystic, dizoic exogenous oocysts (Megía-Palma et al., 2015). However, because of their importance to domestic and companion animal health, members of the family Eimeriidae have attracted substantially more attention in endothermic animals than other coccidian groups and/or poikiloterm hosts (Jirku et al., 2002; Megía-Palma et al., 2015; Abdel-Baki et al., 2020).

Schneider first described the genus *Eimeria* in 1875 from a rodent host and since then, approximately 2,000 species have been described based on the characteristic tetrasporocystic, dizoic exogenous oocyst (Megía-Palam et al., 2015). However, over the last 25 years, the implementation of molecular techniques rapidly advanced the knowledge of the phylogenetic relationships within the family Eimeriidae (Zhao and Duszynski 2001a, b; Jirku et al., 2002; Kvicerova et al., 2008). Recently phylogenetic analyses indicated high specificity of these parasites to their vertebrate hosts (Honma et al., 2007; Power et al., 2009), and show the paraphyly of the genus *Eimeria* (Morrison, 2009).

Based on recently, phylogenetic analysis using nucleotide sequences of the small sub-unit ribosomal RNA, *Eimeria*-like parasites with tetrasporocystic, dizoic exogenous
oocysts that infect reptiles are considered phylogenetically distant and a sister taxon to the Eimeriidae that infect birds and mammals (Jirku et al., 2002). Jirku et al. (2002) redefined the genus *Eimeria* (Eimeriidae) as tetrasporocystic, dizoic exogenous oocysts where sporocysts excyst by dissolving the complex of Stieda and substieda bodies. In contrast, *Eimeria*-like parasites with tetrasporocystic, dizoic exogenous oocysts infecting lizards and other reptiles do not contain sporocysts with Stieda and substieda bodies, but instead their sporocyst contain a meridional suture (Jirku et al., 2002). However, the relationships among these *Eimeria*-like parasites of reptiles remain unresolved because few species are included in recent phylogenetic studies and little to no information is available on their endogenous development for most *Eimeria*-like coccidia infecting reptilian hosts (Jirku et al., 2002, 2009a, b; Megía-Palma et al., 2015; Abdel-Baki et al., 2020; see below).

Recent but limited studies on the oocyst morphology, and documentation of endogenous development along with sequence and/or phylogenetic data suggest that tetrasporocystic, dizoic oocyst of lizards belong to at least three genera (Jirku et al., 2002, 2009a, b; Megía-Palma et al., 2015; Abdel-Baki et al., 2020). The genus *Choleoeimera* is identified based on having an oocyst OSI shape index (length width ratio) of 1.5-1.8, but always above 1.4 (Paperna and Landsberg, 1989a). Importantly *Choleoeimeria* species develop in the gall bladder and biliary epithelium surface; and during endogenous development, biliary epithelial cells become hypertrophic and are displaced to the surface of the epithelial layer, which has been defined as epicytoplasmic development by Paperna and Landsberg (1989a). In addition to *Choleoeimeria*, at least two other genera of coccidia with tetrasporocystic, dizoic oocysts infect lizards: including *Acroeimeria* and
an undescribed genus of *Eimeria*-like coccidia *incertae saedis*. However, distinguishing between the oocysts of *Acroeimeria* species and the undescribed *Eimeria*-like coccidia *incertae saedis* at the genus level is problematic and the OSI for *Acroeimeria* and *Eimeria*-like coccidia *incertae saedis* overlap (<1.25). Although both genera have been reported to infect and develop in the epithelial cells of the intestine of their lizard hosts, species of *Acroeimeria* are enclosed in the microvillous border of the host cell, where they expand into the intestinal lumen in typical epicytoplasmic development. In contrast, *Eimeria*-like coccidia *incertae saedis* endogenous development is intracytoplasmic and without epithelial cells becoming hypertrophic and displaced to the surface of the epithelial layer of the intestine (Paperna and Landsberg, 1989a, 1989b; Modry and Jirku, 2006).

Importantly, very few species of *Choleoeimeria* and *Acroeimeria* have been sequenced and there is no information on the endogenous development of many assumed *Choleoeimeria* and *Acroeimeria* species (Abdel-Baki et al., 2020, Duszynski et al., 2000). Because many of these species were originally described using strictly oocyst morphology as *Eimeria*, it is not clear if species with oocyst OSI of equal to or less than 1.4 belong to *Acroeimeria* with epicytoplasmic development or the undescribed genus of *Eimeria*-like coccidia with intracytoplasmic development. In fact, no sequence data is available for any *Eimeria*-like coccidian infecting lizards with intracytoplasmic development.

Additionally, most of the available sequence and/or endogenous developmental data on *Eimeria*-like coccidia of lizards are from coccidian species infecting various species of geckos, including species of *Hemidactylus* (Jirku et al., 2002; Megfa-Palma et
al., 2015; Abdel-Baki et al., 2020). Therefore, these species are ideal to begin obtaining total evidence (oocyst morphology, endogenous development, and DNA sequences) data sets to be able to resolve the current taxonomical and phylogenetic problems of Eimeria-like coccidia of lizards.

In this study, I evaluate coccidia infections in two species of introduced geckos (Hemidactylus turcicus and H. mabouia) and collected from Oklahoma and Florida, respectively. Most of the work reported below deals with the Eimeria-like parasites with tetrasporocystic, dizoic exogenous oocysts that infect these two species of lizards. However, I also document an Isosporan coccidian that infected H. mabouia. Specifically, I document oocyst morphology and when possible, the endogenous development and provide sequence data for coccidian species from four genera that infect these geckos. In addition, I review the literature for all reports of coccidian infections in Hemidactylus species. Finally, I use oocyst morphological, endogenous development and DNA sequence data to explore phylogenetic relationships among the coccidia of Hemidactylus species, including providing the first molecular evidence for Eimeria-like coccidian incertae sedis infecting lizards.

MATERIALS AND METHODS

Gecko collection and processing

Sixteen Mediterranean house geckos, Hemidactylus turcicus, were collected at night from the campus of Oklahoma State University, Stillwater (OSU), Payne County, Oklahoma (36°07′20.6″N 97°04′17.3″W) on July 26, 2020 and April 12, 2021 and 28
tropical house geckos, *Hemidactylus mabouia* were collected at night from the campus of Florida Southern College (FSC), Lakeland, Polk County, Florida (28°1'56.6"N 81°56'42"W) on April 1, 2021 and November 18, 2021. All geckos were captured by hand, brought back to the laboratory at OSU or FSC respectively, and individually isolated in 500-ml polypropylene plastic containers with lids for up to 12 hrs or until geckos defecated. Individual gecko fecal samples were placed in a solution of 2.5% potassium dichromate (K$_2$Cr$_2$O$_7$), in caped 1 dram glass vials and stored at room temperature. Samples were evaluated for the presence of coccidia oocysts within 12 hours of fecal collections (see below). All geckos were then identified using morphological keys in Krysko and Daniels (2005), and representative infected geckos with various species of coccidia and representative uninfected control geckos were euthanized by decapitation, and necropsied within 12 hours of being collected. Depending on infection status and oocyst morphology, the entire digestive track including the esophagus, stomach, liver and gall bladder and small and large intestines of each infected gecko and a few uninfected control geckos were either fixed in Bouin’s fixative for histological analyses (see below) or in 100% ethanol for DNA extraction (see below). Gecko carcasses were stored in glass vials in 100% ethanol. Prevalence was calculated according to Bush et al. (1997) for each coccidian species/morphotype for each gecko species.

**Oocyst and endogenous parasite development processing**

Potassium dichromate wet mounts with coverslips of all fecal samples were examined for oocyst using an Olympus BX–51 upright research microscope (Olympus, Tokyo, Japan) configured for bright field and Nomarski differential interference contrast
(DIC) microscopy with plain fluorite objectives at 400× to 1000× total magnification. Measurements of at least 30 oocysts for each coccidial morphotype were taken from captured digital images using an Olympus 5-megapixel digital camera and ImageJ software (Schneider et al., 2012) and following the guidelines for oocyst morphology of Duszynski and Wilber (1997).

To document endogenous development, digestive tracks fixed in Bouin’s fixative from geckos with single coccidial morphotype infections were divided into five regions. These include the (1) esophagus and stomach containing the liver and gall bladder, the (2) anterior, (3) middle, and (4) posterior regions of the small intestine, and the (5) large intestine. Each sample was then dehydrated in a graded series of ethanol, cleared in xylene, embedded in paraffin, and sectioned at 6 µm using a ThermoFisher Scientific HM 325 Rotary Microtome (Waltham, Massachusetts). Sections were then affixed to slides, stained with hematoxylin and eosin, mounted in Canada balsam, and examined microscopically following protocols in Bolek et al. (2003). When present, an attempt was made to measure at least 10 schizonts, microgamonts and/or macrogamonts for each coccidial morphotype. Endogenous development for each coccidial morphotype was classified as epicytoplasmic or intracytoplasmic (Paperna and Landsberg, 1989b). More specifically, epicytoplasmic development was defined as either infected biliary epithelial cells that are hypertrophic and expand into the lumen of the gall bladder (Choleoeimeria) or intestinal epithelial cells with endogenous stages enclosed in the microvillous border of the host cell that expand into the lumen of the intestine (Acroeimeria). In contrast, intracytoplasmic development (Isospora or Eimeria-like coccidia incertae sedis) included coccidia species that develop either within the nucleus (Isospora sp.) or in the
cytoplasm of intestinal epithelial cells (*Eimeria*-like coccidia *incertae saedis*) but never expand into the intestinal lumen (Paperna and Landsberg, 1989b; Jirku et al., 2002). All measurements of oocysts and endogenous stages are in micrometers (µm) and are reported as an average followed by a range.

**DNA Extraction**

To obtain sequence data for specific coccidia morphotypes 3 mm sections of the 100% ethanol fixed digestive tracks from geckos with single coccidian morphotype infections were used for DNA extraction. Briefly, DNA was extracted from ethanol preserved tissue by drying in a Thermolyne dri-bath heated to 56°C for approximately 30 minutes to evaporate ethanol. Then the DNeasy Blood and Tissue Kit (Qiagen, CA) was used following the recommended protocol for tissue samples.

**PCR Amplification and Sequencing**

The 18s ribosomal RNA gene was amplified from extracted DNA samples by PCR using previously published primers (Abdel-Baki et al., 2020; Megía-Palma et al., 2015). The primers that were used were: HepF300: 59-GTT TCT GAC CTA TCA GCT TTC GAC G-39, Hep900 59-C AAA TCT AAG AAT TTC ACC TCT GAC-39 (~600 bp) For the gene target, 25 μL PCR reactions were performed using the Platinum SuperFi II Green PCR Master Mix Kit (Invitrogen, Carlsbad, CA). The PCR program was as follows: Initial denature at 94°C for 3 minutes followed by 35 cycles of 94°C for 20s with an annealing temperature of 60°C for 30s and an extension step of 72°C for 1 minute, and a final extension of 72°C for 10 minutes. PCR success was determined by running 4 µL of PCR product on a 1% agarose gel and only single, solid bands were prepped for sequencing using the Promega Wizard DNA Purification Kit (Promega, WI).
Sanger sequencing of PCR amplicons was performed with the primers on an Applied Biosystems 3730 capillary sequencer at the Oklahoma State University core facility. Forward and reverse chromatograms were aligned and inspected in MEGA XI (Kumar et al., 2018).

**Phylogenetic analyses**

Gene sequences of the 18s rRNA, for *Eimeria*-like parasites from lizards in GenBank and used by Megía-Palma et al. (2015) were combined with more recently sequenced from Abdel-Baki et al. (2020). The alignment was created using the MUSCLE (Edgar, 2004) feature of MEGA 11 (Tamura et al., 2021). Due to limited sequence data availability for this group the final alignment had 19 sequences and 535 positions after gaps were removed and sequences trimmed.

Additionally, due to a relatively short sequence of the 18s gene obtained for *I. hemidactyli* during this study and the previous phylogenetic studies on lizard *Isospora* species by Megía-Palma et al. (2016) and Megía-Palma et al. (2017). I compared the sequence data of *I. hemidactyli* to all previous 18s rRNA *Isospora* sequences from lizards along with closely related genera of coccidians including *Lankesterella* and *Caryospora* from reptiles and amphibians and *Eimeria* species from cranes. Gene sequences of the 18s rRNA, for *Isospora* were obtained from GenBank and an aligned as described above. The final alignment consisted of 30 sequences and 693 positions after gaps were removed and sequences were trimmed. The tree was rooted with *Schellackia* species which infect reptiles and amphibians.

Both phylogenies were estimated using the Maximum Likelihood (ML) framework within MEGA XI (Tamura et al., 2021) and the Gamma model (Hasegawa et
al., 1985) was found to be the best fit using the MEGA XI modeltest feature. Support values of nodes for all trees were assessed using 1000 bootstrap replications. Additionally, pairwise distance tables for all sequence pairs for the lizard Eimeria like coccidia and lizard Isospora phylogenetic analyses were calculated in MEGA XI (Tamura et al., 2021).

RESULTS

Five of 16 (31%) of Mediterranean house geckos, H. turcicus examined from Stillwater, OK, were infected with a single coccidian morphotype/species. Based on oocyst morphology and histological evaluation of endogenous development this coccidian was identified as Acroeimeria liner (McAllister et al., 1988; see below).

Of the 28 tropical house geckos, Hemidactylus mabouia collected from Lakeland, Polk County, Florida 16 (57%) was infected with four coccidian morphotypes/species. Of the four coccidian morphotypes/species, Eimeria i. s. boveroi Carini and Pinto, 1926 was the only coccidian that was always recovered from geckos co-infected with other coccidian species. Co-infections of E. i. s. boveroi, included Choleoeimeria cf. turcicus, Isospora hemidactyli Carini, 1936, and based on oocyst morphology a coccidian identified as either Acroeimeria liner or Eimeria i. s. maboia (Carini, 1938; McAllister et al., 1988). As a result, other than oocyst morphological data (see below), no sequence or endogenous data were collected for E. i. s. boveroi.

Although oocyst morphology and endogenous development of Choleoeimeria turcicus conformed to previous descriptions of this species from the Mediterranean house gecko, the tropical house gecko is a new host record for this coccidian. As a result, and
because no sequence data are available for *C. turcicus* from *H. turcicus* for comparisons with *C. turcicus* from *H. mabouia*, I am taking the conservative approach and referring to this coccidian as *C. cf. turcicus*.

Based on oocyst morphology and previous host records of coccidia infecting *H. mabouia*, nine geckos were shedding oocysts conforming to the oocyst description for *A. lineri* or *E. i. s. maboia* (McAllister et al., 1988; Carini, 1938). However, based on histological observations of intracytoplasmic development during this study and sequence data (see species re descriptions below), oocysts that morphologically resembled *A. lineri* were morphologically, developmentally, and genetically distinct from *A. lineri* with epicytoplasmic development from *H. turcicus*. As a result, I argue below that this coccidian should be placed in *Eimeria* i. s. *maboia* as the samples obtained from Florida tropical house geckos and based on oocyst morphology and endogenous development are most similar to the original description of *Eimeria maboia* from tropical house geckos by Carini (1938). Finally, it was not unexpected to find *I. hemidactyli* infecting the Florida population of tropical house geckos, as this species was originally described from introduced populations of *H. mabouia* from Brazil (Carini, 1936).

Based on the current data and my literature review four species of *Hemidactylus* are reported to be infected with 14 species of coccidia (Table I). More specifically, three species of coccida, *A. lineri*, *C. turcicus*, and *Isospora sakrani* have been reported from *H. turcicus*. Six species of coccidia, *A. lineri*, *C. cf. turcicus*, *Choleoeimeria rochalimai*, *E. i. s. boveroi, E. i. s. maboia* and *I. hemidactyli* have been reported from *H. mabouia*. Four species of coccidia, *Eimeria* i. s. *dixoni, E. i. s. furmani, Isospora frenatus*, and *I. schlegeli* infect *Hemidactylus frenatus* and two species of coccidia, *Choleoeimeria*
flaviviridis, and Isospora knowlesi have been reported from Hemidactylus flaviviridis (Table I). Of the 14 species of coccidia reported from Hemidactylus species, information on the endogenous development is available for only seven species, including data presented in the current study; whereas sequence data are only available for four species (see below).

Below I provide oocyst morphology endogenous development and available sequence data for the five coccidia morphotypes/species identified in these two Hemidactylus species. In addition, I review all the literature on coccidia of Hemidactylus species, including oocyst morphology, host use, and when known endogenous development and available sequence data.

REDESCRIPTIONS AND DESCRIPTIONS

*Acroeimeria lineri* (McAllister, Upton, and Freed, 1988); Paperna and Landsberg, 1989b

(Fig. 1)

**Synonym:** Eimeria lineri McAllister, Upton and Freed, 1988.

**Type host:** Hemidactylus turcicus (Linnaeus, 1758).

**Other hosts:** Hemidactylus mabouia (Moreau de Jonnès, 1818).

**Type locality:** Harris County, Texas and Terrabonne Parish, Louisiana (McAllister et al., 1988).

**Geographic distribution:** From *H. turcicus*: Stillwater, Oklahoma (this study); St. Francis County, Arkansas and Union County, Arkansas (McAllister et al., 2017); Israel
(Paperna and Landsberg, 1989b); South-west Turkey (Daszak and Ball, 1991); Giza, Egypt (Abdel-Baki et al., 2020; but see below). From *H. mabouia*: Pretoria, South Africa (Paperna and Landsberg, 1989a).

**Prevalence:** In *H. turcicus*: 31% (5/16) Stillwater, Oklahoma (this study); 77.3% (17/22) Harris County, Texas (McAllister et al., 1988); 42.9% (3/7) Terrabonne Parish, Louisiana (McAllister et al., 1988); 100% (1/1) St. Francis County, Arkansas (McAllister et al., 2017); 67% (2/3) Union County, Arkansas (McAllister et al., 2017); 54% (15/28) Israel (Paperna and Landsberg, 1989a); 9% (1/11) South-west Turkey (Daszak and Ball, 1991); 30% (3/10) Giza, Egypt (Abdel-Baki et al., 2020); In *H. mabouia*: 100% (2/2) Pretoria, South Africa (Paperna and Landsberg, 1989b).

**Description of sporulated oocyst:** The oocyst contains 4 sporocyst with 2 sporozoites per sporocyst. The oocyst is ellipsoid in shape and measures 25.6 x 18.8 (23-30 x 14-22); oocyst shape index (OSI) (length/width) is 1.4 (1.1-1.7). The oocyst has a smooth, bilayered wall. The micropyle and oocyst residuum are absent. Oocyst size and OSI is relatively similar across populations and geographical area, with average oocyst OSI, length and width reported as 1.3-1.5, 24-25 and 16-20 respectively (Table I); except for distinctly smaller OSI and average oocyst length of 1.2 and 22.5 reported for populations of *A. liners* from *H. turcicus* collected from Egypt (Abdel-Baki et al., 2020; Table I). When reported, a polar granule is present in some oocysts (this study; McAllister et al., 1988) or all oocysts (McAllister et al., 1988; McAllister et al., 2017) in some populations but absent from all oocysts in other populations (Paperna and Landsberg, 1989b; Abdel-Baki et al., 2020).
Description of sporocyst and sporozoites: The sporocyst are ellipsoid and measure 8.4 x 7.4 (7-10 x 6-9); shape index 1.1 (0.8-1.3). Sporocyst size and L/W ratio is relatively similar across populations and geographical area, with average sporocyst L/W ratios, and length and width reported as 1.1-1.3, 7.4-9.9 and 6.2-7.8 respectively (Table I). Sutures in the sporocyst open to form a longitudinal slit. Stedia and substedia bodies are absent. Sporocyst residuum is present and is composed of numerous granules. The sporozoites are arranged head to tail in the sporocyst; and contain one spheroidal RBV at each end and a central N.

Endogenous stages: Endogenous development has been documented for A. linieri from H. turcicus populations from Israel, Egypt, Arkansas and Oklahoma, U.S.A and from H. mabouia from South African (see Table I). Endogenous development is reported as epicytoplasmic in the intestinal cells of H. turcicus from Stillwater, Oklahoma, St. Francis and Union County, Arkansas, and populations in Israel (McAllister et al., 2017; Paerna and Landsberg, 1989b); and from H. mabouia from South Africa (Paerna and Landsberg, 1989b). Importantly all but one histological study from a H. turcicus populations from Egypt (see below) clearly demonstrate that the endogenous stages are enclosed in the microvillous border of host intestinal cells which always expand into the intestinal lumen (this study; Paperna and Landsberg, 1989b; McAllister et al., 2017). In contrast, endogenous development was reported to include both epicytoplasmic and intracytoplasmic development from populations of H. turcicus from Egypt (Abdel-Baki et al., 2020; see below). In this study meronts with developed merozoites from H. turcicus populations in Stillwater, Oklahoma measured 12.5 x 9.8. Each meront contains many merozoites. Microgamonts measured 14.5 x 11.7 (9.9-19.8 x 6.8-16.1). The
microgamonts contained numerous nuclei. Macrogamons measured 23.1 x 17.7 (15.6-34.9 x 10.2-26.6). The macrogamonts contained a distinct nucleus. These measurements were similar to previous reports of endogenous development of *A. liner* (see Table I).

*Sporulation:* Exogenous, the oocyst sporulate in the feces and lumen of the digestive track after oocysts exit host intestinal epithelial cells (this study; Paperna and Landsberg, 1989b).

*Prepatent and patent periods:* Unknown.

*Site of infection:* Endogenous stages have been reported from epithelial cells of the middle and posterior region of the small intestine of *H. turcicus* from Stillwater, Oklahoma (this study); from the anterior and/or middle region of the small intestine of *H. turcicus* from Texas and Louisiana U.S.A., and Israel (McAllister et al., 1988; Paperna and Landsberg, 1989b); the anterior and middle region of the small intestine of *H. mabouia* from South Africa (Paperna and Landsberg, 1989b) and an undisclosed location in the small intestine from populations of *H. turcicus* from Egypt (Abdel-Baki et al., 2020).

*Pathology:* Unknown.

*Life cycle studies and host specificity:* Unknown.

*Molecular data:* Partial 18s rDNA gene sequence (535 bp) from *H. turcicus*, Stillwater, Oklahoma (Pending GenBank accession No.; partial 18s rDNA gene sequence (535 bp) from *H. turcicus*, Stillwater Oklahoma (Pending GenBank Accession No.; this study). Partial 18s rDNA gene sequence (632 bp) from *H. turcicus*, Abu Rawash Giza, Egypt (GenBank accession No. MN733371; Abdel-Baki et al., 2020; but see below).
Material deposited: Syntypes (oocysts in 10% formalin) are deposited in the U.S. National Helminthological Collection, Animal Parasitology Institute, U.S. Department of Agriculture, Beltsville, Maryland 20705, as USNM Helm. Coll. No. 80260. Voucher specimens of *H. turcicus turcicus* from Houston, Harris Co., Texas, and Houma, Terrabonne Parish, Louisiana, U.S.A. are deposited in the Arkansas State University Museum of Zoology, State University, Arkansas (ASUMZ 8535-8541, 8649-8665, 8667-8673; McAllister et al., 1988). Photomicrographs of oocysts from *H. turcicus* populations from El Dorado, Union Co., Arkansas (HWML 139319); a host photovoucher was deposited in the Arkansas State University Museum of Zoology Herpetology Collection, as ASUMZ 33619 (McAllister et al., 2017). Photosyntypes of the sporulated oocysts and histological sections of the small intestine sections were deposited in the parasitological collection of the Zoology Department Museum, College of Science, King Saud University, Saudi Arabia, with accession number Ac/01/2019; partial 18S rDNA consensus sequence was deposited in GenBank under accession number MN733371 (Abdel-Baki et al., 2020; but see below). Photomicrographs of oocysts and histological sections of samples from *H. turcicus* from Stillwater, Oklahoma and this study will be deposited in the Harold W. Manter Laboratory Collection (HWML); in addition, vouchers of *H. turcicus* hosts will be deposited in the Collection of Vertebrates (COV) at Oklahoma State University.

Remarks: *Acroeimeria linerl* has been reported from two species of house geckos, including *H. trucicus* and *H. mabouia* and from multiple locations across the world. However, Abdel-Baki *et al.* (2020) examined *A. linerl* from *H. turcicus* collected in Egypt and compared their findings to all previous studies of this *A. linerl*. They suggest
that based on inconsistencies in endogenous development and variation in reported oocyst shapes of *A. lineri* among various studies this coccidian could represent multiple species (McAllister et al., 1988; Paperna and Landsberg, 1989a; Paperna and Landsberg, 1989b; McAllister et al., 2017; Abdel-Baki et al., 2020). Although there is some variation in oocyst size of *A. lineri* based on host species and location most average oocysts length and width measurements fall in the range of 24.1-25.6 µm (Table I), with average oocyst lengths from *H. turcicus* collected from Egypt being smaller (20.5 µm) than reports of oocyst measurements for all other reports of *A. lineri* from *H. turcicus* or *H. mabouia* (Table I; see discussion in Abdel-Baki et al., 2020). More importantly, the endogenous development of *A. lineri* from Mediterranean house geckos from Egypt, was also distinct from the current and previous reports of endogenous development for this coccidian from *H. turcicus* from Oklahoma and Arkansas U.S.A., and Israel and from *H. mabouia* from South Africa (Abdel-Baki et al., 2020; Paperna and Landsberg, 1989b; McAllister et al., 2017; this study). Abdel-Baki et al., (2020) indicated that they observed both intracytoplasmic and epicytoplasmic development of *A. lineri* within epithelial cells of the small intestine of *H. turcicus*; whereas three other endogenous studies of *A. lineri* from *H. turcicus* or *H. mabouia* only observed epicytoplasmic development and including in this study (Paperna and Landsberg, 1989b; McAllister et al., 2017; this study).

Although Abdel-Baki *et al.* (2020) observed intracytoplasmic development of what they identified as *A. lineri* (see Fig. 2 Abdel-Baki et al., 2020); it is less clear they observed epicytoplasmic development (see Fig. 3; Abdel-Baki et al., 2020). Paperna (1989) and Paperna and Landsberg (1989b) originally defined epicytoplasmic
development for eimerid coccidians of lizards that develop within the microvillar zone of the gut epithelium. More specifically the parasites are enclosed in parasitophorous envelopes within its host cells, and bulge above the epithelial layer into the intestinal lumen. This was clearly observed for *A. lineri* from *H. turcicus* from Oklahoma (this study), Arkansas (McAllister et al., 2017) and Israel (Paperna and Landsberg, 1989b) and in *A. lineri* infecting *H. mabouia* from South Africa (Paperna and Landsberg, 1989b). In contrast the few occasions where Abdel-Baki et al. (2020) observed macrogamonts or microgamonts above the epithelial layer of the intestinal lumen and what they argued demonstrated epicytoplasmic development (Fig. 3 in Abdel-Baki et al., 2020), commonly occurred next to epithelial cells with tears suggesting potential artifacts that occurred during tissue sectioning, and these infected cells did not distinctly bulge above the epithelial layer into the intestinal lumen.

Based on endogenous development, oocyst morphology and partial 18s rDNA sequences (see below) the Abdel-Baki et al. (2020) *A. lineri* samples from *H. turcicus* from Egypt are more similar in oocyst size, endogenous development, and sequence similarity to *E. i.s. maboia* samples from *H. mabouia* from Florida obtained during this study (see below; Table I; Fig. 6). Importantly the sequence for *A. lineri* from Egypt nested within a distinct clade from *Acroeimeria* and *Choleoeimeria* and together with sequences generated in this study for *E. i. s. maboia* with intracytoplasmic development from *H. mabouia* from Florida (see below) and a sequence of *Eimeria tokayae*; whereas the sequence of *A. lineri* from *H. turcicus* and also generated in this study was nested within a clade including *A. sceloporis* also with distinctly epicytoplasmic development and with *A. tarentolae* and *A. tropidurid* for which endogenous development has not been
documented. The clear differences in endogenous development, sequence divergence, and to a lesser degree oocyst morphology support the hypothesis by Abdel-Baki et al. (2020) that *A. lineri* may represent multiple species of coccidia. More importantly the work presented in this study strongly suggests that oocysts that are morphologically similar to the oocyst morphology of *A. lineri* can represent species from multiple genera and therefore to differentiate species/genera relationships of coccidia from lizards, coccidologists must include morphological, endogenous, and sequence data.

*Choleoeimeria cf. turcicus* (Upton, McAllister, and Freed, 1988); Paperna and Landsberg, 1989a (Fig. 2)


*Type host: Hemidactylus turcicus* (Linnaeus, 1758).

*Other hosts: Hemidactylus mabouia* (Moreau de Jonnès, 1818).

*Type locality:* Houston Zoological Gardens, Harris County, Texas, USA.

*Geographic distribution: In H. turcicus:* Harris County, Texas (Upton et al., 1988); Union County, Arkansas (McAllister et al. 2017); Afula, Tel Aviv and Rehovot, Israel (Paperna and Landsberg, 1989a); Giza, Egypt (Abdel-Haleem et al., 2016). In *H. mabouia:* Polk County, Florida (this study).

*Prevalence:* In *H. turcicus:* 21.1% (8/38) Harris County, Texas (Upton et al., 1988); 25% (7/28) Afula, Tel Aviv and Rehovot, Israel (Paperna and Landsberg, 1989a); 30% (3/10) Giza, Egypt (Abdel-Haleem et al., 2016); 33% (1/3) Union County, Arkansas (McAllister et al. 2017). In *H. mabouia:* 14% (4/28) Polk County, Florida (this study).

*Description of sporulated oocyst:* The oocyst has 4 sporocyst with 2 sporozoites per sporocyst. The oocyst is ellipsoidal and elongated in shape. The oocyst has a smooth
bilayered wall. A micropyle, residuum is absent. A polar granule was present in some oocysts. Oocyst measured 40 x 17.4 (28-33.4 x 16- 19); OSI 1.8 (1.4-2.1).

*Description of sporocyst and sporozoites:* Sporocysts measured 10.2 x 8.2 (8-12 x 7-2); Shape index 1.2 (1-1.4). Stedia and substedia bodies were absent in the sporocyst. The 2 sporozoites were arranged from head to tail in the sporocyst.

*Endogenous stages:* The endogenous stages develop in the epithelial cells of the gallbladder. The endogenous development is epicytoplasmic. Meronts with many merozoites measured 9 x 7.6 (5.8-17.5 x 5.3-13.2). Microgamonts with many nuclei measured 16 x 13.4 (12-23.6 x 9.4-18.4). Macrogamonts with a single nucleus measured 19 x 15 (12.7-28 x 9.4-19.2).

*Sporulation:* Endogenous in gall bladder (this study; Upton et al., 1988).

*Prepatent and patent periods:* Unknown.

*Site of infection:* *H. turcicus:* In the epithelial cells of the gall bladder, where infected cells are displaced to the surface of the epithelial tissue (Upton et al., 1988; Paperna and Landsberg, 1989a; Abdel-Haleem et al., 2016; McAllister et al., 2017). In *H. mabouia:* In the epithelial cells of the gall bladder; where infected cells are displaced to the surface of the epithelial tissue (epicytoplasmic development; this study).

*Pathology:* Unknown.

*Life cycle studies and host specificity:* Unknown.

*Molecular data:* Partial 18s rDNA gene sequence (535 bp) from *H. mabouia*, Polk County, Florida (Pending GenBank accession No.).

*Material deposited:* Oocyst in 10% formalin deposited in the US National Museum, Beltsville, Maryland (USNM 79587) (Upton et al., 1988). Photomicrographs
and sections of endogenous development from *Choleoeimeria* will be deposited in the Harold W. Manter Laboratory Collection and *H. mabouia* vouchers from this study will be deposited in the Collection of Vertebrates at Oklahoma State University.

**Remarks:** The morphology of the oocysts *C. cf. turcicus* from *H. mabouia* and oocysts of *C. turcicus* from *H. turcicus* are comparable in morphology (Table I). The measurements of the oocysts from *H. mabouia* from Florida, are 32 x 17.4 (28-33.4 x 16-9) (this study) and overlap in length and width with oocysts from *H. turcicus* from Arkansas, Texas, Egypt, and Israel, (Table I; Upton et al., 1988; McAllister et al., 2017; Paperna and Landsberg, 1989a; Paperna and Landsberg, 1989b; Abdel-Haleem et al., 2016). Abdel-Haleem (2016) reported measurements of 9 (7–10) for meronts, 16 (15–17) for microgamonts, and 27 x 16 (25-29 x 15-18) for macrogamonts which were similar to measurements of endogenous stages found during this study for *C. cf. turcicus* from *H. mabouia* being 9 x 7.6 (5.8-17.5 x 5.3-13.2) for meronts, 16 x 13.4 (12-23.6 x 9.4-18.4) for microgamonts, and 19 x 15 (12.7-28 x 9.4-19.2) for macrogamonts but were substantially different in sizes than these stages reported by Paperna and Landsberg (1989a, 1989b) from *H. turcicus* from Israel. Because of the similarity in oocyst morphology and endogenous development, this coccidian is most likely *C. turcicus*. However, because no sequence data are available for *C. turcicus* from *H. turcicus* for comparisons *C. turcicus* from *H. mabouia*, I am taking the conservative approach and referring to this coccidian as *C. cf. turcicus*. Importantly, the sequence generated for *C. cf. turcicus* in this study clearly places it in the *Choleoeimeria* clade with sequences for four other *Choleoemeria* species from other species of lizards (see below; Fig. 6).

*Eimeria i. s. boveroi* Carini and Pinto, 1926
Type host: Hemidactylus mabouia (Moreau de Jonnès, 1818).

Other hosts: none.

Type locality: State of São Paulo, Brazil (Carini and Pinto, 1926).

Geographic distribution: H. mabouia: Polk County, Florida (this study); State of São Paulo, Brazil (Carini and Pinto, 1926); Capanema and Belém, State of Pará, Brazil (Landsberg and Paperna, 1999); Boca del Rio, Veracruz, Mexico (McAllister and Upton, 1989); Cameroon (Upton et al., 1992); Capanema and Belém, State of Pará, Brazil (Lainson and Paperna, 1999); Capanema and Belém, State of Pará, Brazil (Lainson and Paperna, 1999).

Prevalence: From H. mabouia: 14% (4/28) Polk County, Florida (this study); prevalence not given for the type locality in the State of São Paulo, Brazil (Carini and Pinto, 1926); 27% (3/11) Capanema and Belém, State of Pará, Brazil (Lainson and Paperna, 1999); 25% (12/23) Capanema and Belém, State of Pará, Brazil (Lainson and Paperna, 1999); 52% (12/23) 100% (1/1) Boca del Rio, Veracruz, Mexico (McAllister and Upton, 1989); 6/15 (40%) Cameroon (Upton et al., 1992).

Description of sporulated oocyst: The oocyst has four sporocysts with two sporozoites per sporocyst. The oocyst is spherical in shape. Oocyst from the Florida population of H. mabouia were 19.7 x 18 (15.4-23.3 x 14.2-21.8) with an OSI of 1.1 (1-1.3) and were similar in size to previous reports of oocyst morphology for this coccidia species reported from Brazil and Mexico (Table I). The oocyst has a smooth bilayered wall. The polar granules are absent.
Description of sporocyst and sporozoites: There are no stedia or substedia bodies. Sporocyst size 8.5 x 7.4 (7-10 x 6-9). Shape index 1.2 (1-1.4) and agrees with previous descriptions from Brazil and Mexico (Table I). The sporozoites are vermiform and arranged head to tail in the sporocyst.

Endogenous stages: Previous studies indicate that endogenous development is intracytoplasmic in epithelial cells of the small intestine with meronts being reported as 4.0 x 1.0 to 8.0 x 5.0; microgamonts measuring 13.0 x 9.0 and macrogamonts measuring 16.0 x 6.0 (Lainson and Paperna, 1999).

Sporulation: Unknown.

Prepatent and patent periods: Unknown.

Site of infection: Epithelial cells of the small intestine (Lainson and Paperna, 1999).

Pathology: Unknown.

Life cycle studies and host specificity: Unknown.

Molecular data: None.

Material deposited: Photomicrographs and sections of endogenous developmental stages will be deposited in the Harold W. Manter Laboratory Collection (this study). A H. mabouia specimen collected from Polk County, Florida (this study) will be deposited as a host voucher in the Collection of Vertebrates at Oklahoma State University.

Remarks: The measurements of the oocyst in H. mabouia from Florida, 19.7 x 18 (15.4-23.3 x 14.2-21.8), are very similar to the measurements reported for oocyst in H. mabouia from Brazil and Mexico (see Table I; McAllister and Upton, 1989; Paperna and Lainson, 1999). Unfortunately, endogenous development could not be documented in this
species and from infected geckos from Florida because all infected geckos were co-infected with other intestinal coccidia species.

**Eimeria i. s. maboia** Carini, 1938.

(Fig. 4)

*Type host:* Hemidactylus mabouia (Moreau de Jonnès, 1818).

*Other hosts:* None.

*Type locality:* State of São Paulo, south Brazil (Carini, 1938).

*Geographic distribution:* Polk County, Florida (this study); State of São Paulo, Brazil (Carini, 1938).

*Prevalence:* 32% (9/28) Polk County, Florida (this study); prevalence not given for the type locality in the State of São Paulo, Brazil (Carini, 1938).

*Description of sporulated oocyst:* The oocyst has 4 sporocysts with 2 sporozoites per sporocyst. The oocysts are ellipsoid in shape and measure 21.6 x 18 (18-24 x 15-20); OSI 1.2 (1.1-1.3) and were similar in size to previous reports from the type locality in Brazil (Table I). The oocyst has a smooth, bilayered wall. The micropyle, oocyst residuum and polar granule are absent.

*Description of sporocyst and sporozoites:* The sporocysts are ellipsoid and measure 8.8 x 7.6 (8-10 x 6-8); shape index 1.2 (1-1.4) and were similar in size to previous reports from Brazil (Table I). Sutures in the sporocyst open to form a longitudinal slit. Stedia and substedia bodies are absent. The sporocyst contains 2 sporozoites that are arranged from head to tail.
**Endogenous stages**: The endogenous stages are in the epithelial cells and in the middle and posterior region of the small intestine. Endogenous development is intracytoplasmic in the epithelial cells of the small intestine. Meronts with merozoites measured 13.2 x 9.7 (7.3-18.3 x 4.8-12.5). Microgamonts contained multiple nuclei and are 11.2 x 6.7 (5.9-17.8 x 5.2-8.3) and were similar in size to previous reports from Brazil (Table I). Macrogamonts have a singular nucleus and measure 19.8 x 13.6 (12.1-26.1 x 9.4-19.8). These measurements are similar to the measurements of meronts and microgamonts provided for this species by Carini (1938) from the type locality in Brazil (Table I).

**Sporulation**: Exogenous, the oocyst sporulate in the feces and lumen of the digestive track after oocysts exit host intestinal epithelial cells.

**Prepatent and patent periods**: Unknown.

**Site of infection**: epithelial cells of the small intestine.

**Pathology**: Unknown.

**Life cycle studies and host specificity**: Unknown.

**Molecular data**: Three partial 18s rDNA gene sequence (535, 535, 535 bp) from *H. mabouia*, Polk County, Florida (Pending GenBank accession No.; this study).

**Material deposited**: Photomicrographs and sections of endogenous development will be deposited in the Harold W. Manter Laboratory Collection. Vouchers of *H. mabouia* and the host will be deposited in the Collection of Vertebrates at Oklahoma State University (this study).

**Remarks**: *Eimeria* i. s. *maboia* oocyst morphology is most similar to oocysts of *A. lineri* (Table I). However, unlike *A. lineri* with epicytoplasmic development, except from
Egypt (Abdel-Baki et al., 2020; see above), which have been reported to have intracytoplasmic and epicytoplasmic development, *E. i. s. maboia* has strictly intracytoplasmic development. Additionally, size and OSI of oocysts of *E. i. s. maboia* from this study and the original description from Brazil by Carini (1938) is most similar to the descriptions of oocyst of *A. lineri* from *H. turcicus* from Egypt (21.6 x 18, 1.2; 20 x 17, 1.21 vs. 22.5 x 20, 1.2). Importantly the sequence for *A. lineri* from Egypt nested together within a clade containing sequences generated in this study for *E. i. s. maboia* from *H. mabouia* from Florida and a sequence of *Eimeria tokayae*; whereas the sequence of *A. lineri* from *H. turcicus* from Oklahoma and generated in this study was nested within a clade of sequences from three species of *Acroeimeria* (see below; Fig. 6). Based on endogenous development, oocyst morphology and partial 18s rDNA sequences the *A. lineri* samples from *H. turcicus* from Egypt (Abdel-Baki et al., 2020), *E. i. s. maboia* from *H. mabouia* from Florida, and *E. tokayae* (Table I; Fig. 6) represent a distinct linage of lizard coccidia distinct from the genera *Acroeimeria* and *Choleoeimeria* (Fig. 6). These phylogenetic data are some of the first that support the well accepted hypothesis that several more species of *Eimeria*-like coccidia from reptiles, which excyst via the suture in the sporocyst wall do not fit neither the emended definition of the Eimeriidae (see discussions in Jirku et al., 2002) nor the definition of the genera *Acroeimeria* or *Choleoeimeria*. These species apparently belong to intestinal species with sporocyst wall sutures with intracytoplasmic development. Unfortunately, so few endogenous stages have been documented from lizard/reptilian coccidia in general and/or linked to sequence data (see below) I take a conservative approach that it is currently unjustifiable to erect a new genus for these species until more data are available for coccidia species from other
genera and species of lizard hosts. However, the work presented here clearly indicates that to differentiate between species and/or genera of lizard coccidia documentation of the endogenous development, along with sequence data are necessary to complement oocyst morphology information.

*Isospora hemidactyli* Carini, 1936

(Fig. 5)

*Type host:* *Hemidactylus mabouia* (Moreau de Jonnès, 1818).

*Other hosts:* None.

*Type locality:* San Paulo, Brazil (Carini, 1936).

*Geographic distribution:* From *H. mabouia*: Polk County, Florida (this study); Capanema and Belém, State of Pará, Brazil (Lainson and Paperna, 1999); Rio de Janeiro, Brazil (Berto et al., 2008); Cameroon (Upton et al., 1992).

*Prevalence:* From *H. mabouia*: 25% (7/28) Polk County, Florida (this study); prevalence not provided for the type location, San Paulo, Brazil (Carini, 1936); 17% (4/23) Capanema and Belém, State of Pará, Brazil (Lainson and Paperna, 1999); 18% (2/11) Capanema and Belém, State of Pará, Brazil (Lainson and Paperna, 1999); 100% (1/1) Rio de Janeiro, Brazil (Berto et al., 2008); 27% (4/15) Cameroon (Upton et al., 1992).

*Description of sporulated oocyst:* The oocyst is ellipsoid in shape with single smooth wall. Oocyst size measures 21 x 19.4 (15.1-25 x 15-22.6); OSI 1.1 (1-1.2). The micropyle is absent. The polar bodies are present. Many but not all oocysts from Florida house gecko populations contain a single polar granule (this study). The oocyst has 2 sporocyst with 4 sporozoites per sporocyst.
Description of sporocyst and sporozoites: Sporocyst size measures 10 x 8.5 (7-9.6 x 7-9.5); Shape index 1.2 (1-1.4). The sporocyst has stedia and substedia bodies. The four sporozoites are arranged head to tail in the sporocyst.

Endogenous stages: The endogenous stages are in the posterior part of the small intestine. The endogenous development is intracytoplasmic in the intestinal cell. The meronts contain many merozoites and measure 10 x 6.8. Microgamonts contain many nuclei and measure 10 x 7 (4-16.2 x 3.3-13.1). Macrogamonts contain one nucleus and measure 18.6 x 11 (10.6-30.7 x 6.8-19.3).

Sporulation: Exogenous after 24 hours outside of the host (Lainson and Paperna 1999).

Prepatent and patent periods: Unknown.

Site of infection: In the epithelial cells in the middle to posterior region of the small intestine (this study; Carini, 1936); in the nucleus of the epithelial cells of the small intestine (Lainson and Paperna, 1999).

Pathology: Unknown.

Life cycle studies and host specificity: Unknown.

Molecular data: Partial 18s rDNA gene sequence (540 bp) from H. mabouia, Polk County, Florida (Pending GenBank accession No.; this study).

Material deposited: Photomicrographs and sections of endogenous development from I. hemidactyli will be deposited in the Harold W. Manter Laboratory Collection. Hemedactilus mabouia vouchers will be deposited in the Collection of Vertebrates at Oklahoma State University (this study). Phototypes, line drawings, and Oocysts in 10% aqueous (v/v) buffered formalin are deposited at the Parasitology Collection, in the
Department of Animal Parasitology, UFRRJ, Seropédica, Rio de Janeiro, Brazil
(repository number 03/2008; Berto et al., 2008).

**Remarks:** The *Isospora* species which infected the small intestine of *H. mabouia* from Polk County, Florida conforms to the description of *I. hemidactylus* found in *H. mabouia* from Brazil (Carini, 1936). The measurements of oocyst from Florida geckos, 21 x 19.4 (15.1-25 x 15-22.6), overlap with oocyst measurements provided for this species from Brazil and Cameroon (Table I; Carini, 1936; Upton et al., 1992; Lainson and Paperna, 1999; Berto et al., 2008). Additionally, the endogenous development of *I. hemidactylus* from Florida populations of *H. mabouia* developed in the nucleus of epithelial cells of the small intestinal cell as reported by Lainson and Paperna (1999) and Paperna and Lainson (2000).

**PHYLOGENETIC ANALYSES**

**Reptilian Eimeria-like Clade**

Five partial sequences of the 18s rRNA gene were generated for three species of lizard *Eimeria*-like coccidia, including three partial sequences for *Eimeria i. s. maboia* (538-541 bp) from *H. mabouia*, one partial 18s sequence for *A. lineri* (533 Bp) from *H. turcicus*; and one partial 18s sequence for *C. cf. turcicus* from *H. mabouia* (526 Bp). The resulting ML phylogeny was rooted with *Toxoplasma gondii*. The phylogeny separates into three clades of lizard coccidia (Fig. 6). One clade consists of *Eimeria*-like coccidia *incertae saedis* species which when known have intracytoplasmic development in the intestines and assumed to have an OSI of less than 1.25. The species in this clade include *E. i. s. maboia, A. lineri* from *H. turcicus* from Egypt, and *Eimeria tokayae* (Fig. 6). The
second clade consists of Acroeimeria species which when known have epicytoplasmic development in the intestines and assumed to have an OSI of less than 1.25. The species in this clade include Acroeimeria tarentolae, A. lineri from H. turcicus from Oklahoma, Acroeimeria sceloporis, and Acroeimeria tropidura (Fig. 6). The third clade consists of Choleoeimeria species which have oocysts with an OSI of greater than 1.4 and when known epicytoplasmic development in the gallbladder. The species in this clade include Choleoeimeria scincorum, Choleoeimeria wiegmanniana, Choleoeimeria gallotiae, Choleoeimeria pogonae, and Choleoeimeria cf. turcicus (Fig. 6).

The uncorrected p distances for the partial 18s rDNA sequence of Eimeria-like coccidia varied from 0-8.6% for the in group (Acroeimeria, Choleoeimeria and Eimeria-like coccidia incertae sedis) and as high as 6.9-13.8% for the outgroup (Table II). Importantly, the uncorrected p distance for the 18s rDNA partial sequences of A. lineri from Oklahoma H. turcicus and the partial 18s rDNA sequence of A. lineri from Egyptian H. turcicus differed by 5.2%. In contrast, partial 18s rDNA sequences of E. i. s. maboia from H. mabouia from Florida were most similar in terms of uncorrected p distance (1.2% difference) to the partial 18s rDNA sequence of A. lineri from H. turcicus from Egypt (Abdel-Baki et al., 2020). Finally, all three 18s rDNA partial sequences of E. i. s. maboia from H. mabouia from Florida differed by 5% from the 18s rDNA partial sequence of A. lineri from Oklahoma H. turcicus in uncorrected p distances.

**Reptilian Isospora Clade**

One partial sequence (534 bp) of the 18s rRNA, for I. hemidactylus from an infected H. mabouia was generated during this study. The resulting ML phylogeny included 10 lizard Isospora species (Fig. 7). In the phylogenetic analysis, the partial 18s
rDNA sequence of *I. hemidactylus* from the Florida *H. mabouia* sample was sister to a partial 18s rDNA sequence of *Isospora gekkonis* and the only other *Isospora* species included in the phylogenetic analysis from a gecko host, sequenced from a captive bread *Phelsuma madagascariensis grandis* (Megía-Palma et al., 2016) and originally described from a population of *P. madagascariensis grandis* from Madagascar (Upton and Barnard, 1987). Importantly, *I. hamidactylus* and *I. gekkonis* were sister to *Caryospora ernsti* from *Anolis carolinensis*, and within a clade with six species of *Lankesterella* from amphibian, bird, and lizard hosts (Fig. 7). Additionally, seven species of *Isospora* from lizard host formed a sister clade, although not well supported, to the *Lankesterella/I. gekkonis/I. hamidactylus/C ernsti* clade with *Isospora wiegmanniana* from *Trogonophis wiegmanni wiegmanni* (Megía-Palma et al., 2016) positioned basally to remaining *Isospora* clades (Fig. 7). Importantly the clade containing *I. hamidactylus* and *I. gekkonis* and *Caryospora ernsti* were the only lizard coccidia included in the phylogenetic analysis that contained a polar granule within their oocysts, none of the other Isosporan species contain a polar granule in their oocysts (Fig. 7). However, oocysts of all snake species of *Caryospora* in a distantly related clade to the lizard *I. gekkonis/I. hamidactylus/C ernsti* clade also contained polar granule within their oocysts (Fig. 7).

The uncorrected p distances for the partial 18s rDNA sequences for the reptilian *Isospora* and other eimereidae coccidian analysis varied from 0.57% between *I. gekkonis* and *I. hemidactylus* to as high as 9% for *L. valsainensis* and *Schellackia bolivari* in the outgroup (Table III).
DISCUSSION

Although in general, the diversity of coccidian parasites in reptilian and more specifically lizard hosts have been largely ignored (Bovee and Telford, 1965; Matuschka and Bannert, 1986; McAllister et al., 2020). There are approximately 6,300 species of lizards, but only a few hundred have been studied for coccidian infections (Duszynski, 2021). However, it is estimated that the coccidian diversity in lizards found across the world is extremely rich. With a conservative estimate of two unique species of coccidia, on average, infecting a unique lizard species, there could be as many as 12,600 species of coccidia that infect lizards across the world (Duszynski, 2021).

Importantly, of the 16 extant families of lizards, the Gekkonidae have been more commonly examined for their coccidia infections than other lizard groups. In fact, no other family of lizards has been studied more thoroughly for coccidian infections. Out of the 62 genera in the family Gekkonidae, 24 genera and 52 species have been examined for coccidia infections (Duszynski et al., 2000).

Currently there are 191 described and numerous undescribed species of Hemidactylus geckos, including as many as 20 putative species-level lineages, of what many currently consider H. mabouia (Agarwal et al., 2021). Out of the 191 described species, only seven species of Hemidactylus have been examined for coccidian infections (Duszynski et al., 2000). These species include Hemidactylus brooki, H. coctoei, H. flaviviridis, H. frenatus, H. mabouia, H. prashadi, and H. turcicus (see Duszynski et al., 2000). Importantly, Hemidactylus geckos are the most surveyed geckos for coccidia, and represent 13% (7/52) of all gecko species examined for this group of intracellular parasites (Duszynski et al., 2000). Of the four species of Hemidactylus geckos that have
records of coccidia, 10 species of coccidians have been reported from these hosts. Based on the current diversity of coccidia in the *Hemidactylus* species examined so far, I estimate there could be as many as 325 species of coccidia infecting geckos of this genus.

As part of this study, I document new locality records for coccidia in the United States, and more specifically from localities in Oklahoma, and Florida. This is the first time that *A. lineri* has been reported from *H. turcicus* in Oklahoma. Additionally, I documented for the first time *C. cf. turcicus, E. i. s. boveroi, E. i. s. maboia* and *I. hemidactyli* from *H. mabouia* in the United States and from Florida. Importantly, *Acroeimeria lineri* reported from *H. turcicus* in Oklahoma and *C. cf. turcicus, E. i. s. boveroi, E. i. s. maboia* and *I. hemidactyli* in *H. mabouia* from Florida are the northern most record for these coccidia species from *Hemidactylus* species in their introduced range (Berto et al., 2008; Abdel-Baki et al., 2020; McAllister et al., 1988; Paperna and Landsberg 1989a; Paperna and Landsberg, 1989b; Paperna and Lainson, 2000; Upton et al., 1988; Carini and Pinto, 1926; Carini, 1938; Lainson and Paperna, 1999; McAllister and Upton, 1989; McAllister et al. 2017; Lainson and Paperna, 1999b). As typical for most related eimerid like coccidia with known life cycles, including other lizard coccidia (Roberts et al., 2013; Walden and Mitchell, 2021) it is assumed that all *Acroeimeria, Choleoeimeria and Eimeria*-like coccidia *incertae saedis* species from lizard hosts have direct life cycles. As a result of this parasite life history characteristic and transmission strategy, it is assumed to be easier for these parasites, and compared to parasites with multiple hosts in their life cycles, to move with their gecko hosts as they move northwards (Fayer, 1980). As the geckos move further north, the coccidia move with their
host which results in new northernmost records as the geckos increase their north range. It is still unclear how and what type of environmental conditions outside of their hosts are needed for these coccidian species to remain infective to their gecko hosts. However, given that all these coccidian species have a relatively thick oocyst walls, a common characteristic for reptilian coccidia (Duszynski et al., 2000), it is assumed that upper and lower temperature limits and not humidity will be important in determining their distribution range compared to their gecko hosts.

In addition, it is currently unclear if other native species of lizards will be susceptible to these coccidian species. It has been hypothesized that most coccidian species of amphibians and reptiles are genus and or family specific, however, currently no experimental host specificity studies have been conducted on coccidian parasites of amphibian and reptiles in general and Hemidactylus species specifically (Bolek et al., 2003; Duszynski et al. 2007; Duszynski et al., 2000; Duszynski et al., 2021). As a result, it will be critical to continue monitoring both native and introduced liard species for their coccidia infections.

**Reptilian *Eimeria*-like phylogenetic relationships**

With the updated phylogenetic analyses of the reptilian *Eimeria*-like clade in the present study, I provide strong preliminary molecular and morphological evidence for a new genus of coccidia in lizards that is distinct from *Acroeimeria* and *Choeloeimeria*. *Eimeria i. s. maboia* found in *H. mabouia* from Florida has tetrasporocystic, dizoic oocysts but intracytoplasmic development in the small intestine and unlike the epicytoplasmic development within the genera *Choeloeimeria* and *Acroeimeria*. Importantly, these three genera with epicytoplasmic development in the small intestine
(Acroeimeria), or gallbladder (Choleoeimeria) or intracytoplasmic development in the small intestine (Eimeria-like coccidia incertae saedis) show a distinct relationship as three distinct clades on the 18s rRNA phylogeny (Fig. 6). Based on the oocyst morphology, endogenous development, and phylogenetic analyses, there is growing evidence of a distinct genus of coccidia that infects lizards and it is currently recognized as Eimeria-like coccidia incertae saedis with distinctly intracytoplasmic development (Paperna and Landsberg, 1989a; 1989b).

There are several more species of Eimeria-like coccidia from reptiles, which excyst via the suture in the sporocyst wall. These taxa fit neither the emended definition of the Eimeriidae (Jirku et al. 2002) nor the definition of the genera Choleoeimeria or Acroeimeria (Paperna and Landsberg, 1989a; 1989b). More specifically, epicytoplasmatic development was defined as either infected biliary epithelial cells that are hypertrophic and expand into the lumen of the gall bladder (Choleoeimeria) or intestinal epithelial cells with endogenous stages enclosed in the microvillous border of the host cell that expand into the lumen of the intestine (Acroeimeria). In contrast, intracytoplasmic development (Eimeria-like coccidia incertae saedis) included coccidia species that develop in the cytoplasm of intestinal epithelial cells but never expand into the intestinal lumen of the intestine (Paperna and Landsberg 1989b; Jirku et al. (2002). Even with the current data and definitions, it is difficult to describe this new genus for Eimeria-like coccidia with intracytoplasmic development because there is only one clear example that includes histological and sequence data. Therefore, in this study I take a conservative approach that it is currently unjustifiable to erect a new genus for these
species until more endogenous developmental data with concurrent sequence data are available for coccidia species from other genera and species of lizard hosts.

**Reptilian Isospora phylogenetic relationships**

Over 100 species of *Isospora* have been described from reptiles (Megía-Palma et al., 2016). Of those, 88 species are known from the suborder Sauria, with the highest diversity (30 species) reported from the family Gekkonidae (Liu et al., 2021). However, as with the *Eimeria*-like coccidia from reptiles, few species of *Isospora* which infect reptiles are included in recent phylogenetic studies and little to no information is available on their endogenous development (Megía-Palma et al., 2016; Lui et al., 2021; Walden and Mitchell, 2021). In fact, including the newly generated partial 18s rDNA sequence generated for *I. hemidactyli* only 11 partial 18srDNA sequences are available for 10 *Isospora* species infecting lizards.

As in all previous phylogenetic studies on Isosporan species from lizard host (Megía-Palma et al., 2016; Megía-Palma et al., 2017; Lui et al., 2021), my work indicates that *Isospora* species from lizards are not a monophyletic group, and suggests a paraphyletic origin of *Isospora* species infecting lizard hosts. My phylogenetic analysis indicated three clades containing *Isospora* species from lizard hosts (Fig. 7). Seven species of *Isospora* from lizard hosts formed a well-supported clade. However, a second clade, although not well supported, and positioned sister to the well supported clade of seven lizard *Isospora* species, includes six species of *Lankesterella* from amphibian, bird, and lizard hosts, and two *Isospora* and one *Caryospora* species from two species of geckos and the green anole, *Anolis carolinensis*, respectively. Finally, *Isospora wiegmanniana* from the checkerboard worm lizard, *Trogonophis wiegmanni wiegmanni*
(Megía-Palma et al., 2016) was positioned basally to the two clades mentioned above and containing species of *Isospora* infecting lizard hosts (Fig. 7).

Importantly, the partial 18s rDNA sequence of *I. hemidactylus* generated in this study was sister to a partial 18s rDNA sequence of *Isospora gekkonis* and the only other *Isospora* species available from another gecko host, the Madagascar giant day gecko, *Phelsuma madagascariensis grandis* (Upton and Barnard, 1987; Megía-Palma et al., 2016). As mentioned above, *I. hemidactylus* and *I. gekkonis* were sister to *Caryospora ernsti* from *Anolis carolinensis*, but nested within a clade of six species of *Lankesterella* from amphibian, bird, and lizard hosts (Fig. 7). This relationship is quite surprising as the oocysts of *Lankesterella* species never exit the vertebrate definitive host, but instead sporulate in the tissue of their definitive vertebrate host. Once sporulated, the sporozoites exit the oocyst and migrate to the blood stream where they are ingested by arthropod or leech intermediate hosts. Within these invertebrate intermediate hosts, the sporozoites mature to the infective stage and are eventually injected into a vertebrate definitive host during blood feeding by the vector host, and where merogony, gamogony and sporogony occur (Barta et al., 2001).

Importantly and as mentioned above, the clade including *I. hemidactylus, I. gekkonis* and *Caryospora ernsti* were the only lizard coccidia included in the phylogenetic analysis that contained a polar granule within their oocysts, whereas none of the other *Isospora* species contain a polar granule in their oocysts (Fig. 7). Although these three species were nested within a clade of *Lankesterella* species, no polar granules have been reported from the oocysts of any *Lankesterella* species, however, oocysts of *Lankesterella* species contain a unique structure of one to several residual bodies
bounded by a coat of electron-dense droplets within the sporulated oocyst (Lainson and Paperna, 1995).

Although no other Isospora species included in the current phylogenetic analysis contained polar granules or oocyst residua, oocysts of Caryospora species from snake hosts which were positioned basally to all other Isospora species and in a distinct clade to the lizard I. gekkonis/I. hamidactylus/C ernsti clade also contained polar granule within their oocysts (Fig. 7). It is currently unclear if and what the differences may be between polar granules and residual bodies within the oocysts of these different genera of coccidia. Clearly and in order to resolve these issues, it will be critical to include sequences of morphologically diverse Isospora species that represent species with various oocyst characteristics from lizard and other reptilian hosts in future phylogenetic studies on reptilian Isopoda and other closely related genera of coccidia.

Conclusion

This study documents new coccidia host and distribution records from two species of introduced house geckos in the United States of America. In addition, I provide new information on the phylogenetic relationships of four genera of lizard coccida using a total evidence approach including information on oocyst morphology, but also endogenous development and sequence data. The data clearly indicates that more work will be required to resolve the phylogenetic relationships among these coccidia genera. In order to get a better understanding of their evolutionary relationships and when considering that estimates suggest over 12,600 species of coccidia that infect lizards across the world, it will be critical to increase our sampling efforts and include information on the oocyst morphology, sequence data and endogenous development for
coccidia from multiple species/families of lizards across the world. And this study is one of the first to add to this effort.
Figure 2.1 Photomicrographs of sporulated oocysts, sporocyst, sporozoites and endogenous stages of *Acroeimeria lineri* (A-I). Oocyst of *Acroeimeria lineri*. Note, unsporulated oocyst (A); two sporozoites arranged head to tail in the sporocyst (C); polar granule (J). Photomicrograph of a cross section of the posterior region of the small intestine from *Hemidactylus turcicus* (K). Endogenous development of *Acroeimeria lineri* on the intestinal villi (L). Note the large number of endogenous stages on the villi (arrows) in the small intestine (M). Schizont (N). Microgamont (O). Macrogamont (P).
Figure 2.2 Photomicrographs of sporulated oocyst, sporocyst, sporozoites and endogenous stages of *Choleoeimeria* cf. *turcicus* (A-C). Oocyst with four sporocyst (A). Oocyst of *Choleoeimeria turcicus* with a polar granule (B). Two sporozoites with refractile bodies (C). Photomicrographs of a section of the gallbladder wall of an infected *Hemidactylus mabouia* (D-E). Note the infected biliary epithelial cells that are hypertrophic and expand into the lumen of the gall bladder. Microgamont (D). Meronts (E). Macrogamont (F).
Figure 2.3 Photomicrographs of sporulated oocyst, sporocyst, and sporozoites of *Eimeria i. s. boveroi* (A-E). Four sporocyst in the oocyst (A). Sporozoites arranged head to tail in the sporozoite (E).
Figure 2.4 Photomicrographs of sporulated oocyst, sporocyst, sporozoites and endogenous stages of *Eimeria* i. s. *maboia*. (A-E). Four sporocyst in the oocyst (A). Sporozoites arranged head to tail in the sporocyst (D). Polar granule (arrow) in the oocyst (E). Photomicrographs of a posterior section of the small intestine from *Hemidacculus mabouia* (F-H). A cross section of the small intestine (F). Endogenous development in the microvilli of the small intestine (G-H). The development is intracytoplasmic (arrows) in the intestinal cells (H). Meronts (I). Microgamonts (J). Macrogamonts (K).
Figure 2.5 Photomicrographs of sporulated oocyst, sporocyst, sporozoites, and endogenous stages of *Isospora hemidactyli* (A-E). Sporocyst with stedia and substedia bodies (A). Polar granule (arrow) (C). Sporozoites arranged head to tail in the sporocyst (D). Photomicrographs of a posterior section of the small intestine from *Hemidactylus mabouia* (F-G). Microgamont (F-H). Microgamont (Left) and Macrogamont (Right) (G).
Figure 2.6 Maximum Likelihood phylogeny of the partial 18s rRNA gene (535 positions, -Ln = -1610.24) using the Gamma model in MEGA XI (Tamura et al., 2021). Values at nodes represent bootstrap support values, assessed with 1000 replications. The phylogeny is rooted with Toxoplasma gondii and Gossia species and shows three Eimeria-like clades infecting lizard hosts. Importantly, and when known, clade one (red) included Eimeria-like coccidia incertae saedis with intracytoplasmic development in the intestines, clade two (green) included Eimeria-like coccidia with epicytoplasmic development in the intestines (defined as Acroeimeria), and clade three (blue) include Eimeria-like coccida with epicytoplasmic development in the gallbladder (defined as Choleoeieria). The check marks represent sequences for coccidia species with known endogenous development, whereas the question marks represent sequences for coccidia species without known endogenous development data.
Figure 2.7 Maximum Likelihood phylogeny of the partial 18s rRNA gene (693 positions, -Ln = -2191.31) using the Gamma model in MEGA XI (Tamura et al., 2021). Values at nodes represent bootstrap support values, assessed with 1000 replications. The phylogeny is rooted with Schellackia species and shows two Isospora clades (green and red brackets) infecting lizard hosts with I. wiegmanniana (blue arrow) basal to the other lizard Isosporan species. Oocyst morphology showing polar granules (black dot free in the oocyst) or the lack of them for all Isospora and Caryospora species from reptiles in the phylogenetic analysis, is mapped on the tree, and as is host group (lizards, snakes, birds and anurans) for all coccidian species included in the tree. Note that in addition to the paraphyletic nature of Isospora from lizards, similarly the position of the genus Caryospora (green and black brackets) indicates it is not monophyletic.
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<th>L/W Ratio</th>
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<th>L/W Ratio</th>
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<th>Meront Size</th>
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60
Table II. Calculated uncorrected p distances using a partial 18s rDNA gene for *Eimeria*-like coccidia from lizards.

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<td>13. <em>C. pogona</em></td>
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<td>14. <em>C. scincorum</em></td>
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<td>15. <em>G. balatonicula</em></td>
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<td>17. <em>G. vargai</em></td>
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<td>18. <em>G. desseri</em></td>
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* Based on partial 18srDNA of *A. lineri* from *H. trucicus* from Egypt.
| Species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| *I. fahdii* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphihabierti* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphibius* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphipus* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| *I. amphiolitii* | 0.04 | 0.04 | 0.02 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
CHAPTER III

CONCLUSION

Coccidiosis is caused by oocyst-forming, single-celled obligate intracellular parasites that infect most phyla of invertebrates and all classes of vertebrates. The disease is recognized as the major health hazard in domestic animal husbandry, in zoo environments, and in wild animal populations (Roberts et al., 2013). However, other than domestic and companion animals, most vertebrate groups in general and lizards more specifically have been ignored for their coccidia diversity. There are approximately 6,300 species of lizards in 40 families, but only a few hundred species from only 16 lizard families have been evaluated for coccidia infections. Even with the small number of lizard species examined, it is estimated that the coccidian diversity in lizards is extremely rich, with a conservative estimate of on average two unique species of coccidia per lizard species, suggesting as many as 12,600 species (Duszynski, 2021). As a result, we know very little about the diversity, host parasite relationships, and phylogenetic relationships of coccidia parasites that infect reptiles in general and lizards specifically.

In this study I examined two species of invasive geckos for their coccidia infections, and I attempted to use a total evidence approach (oocyst morphology, endogenous development, and DNA barcoding) to describe the taxonomical and phylogenetic relationships of four common genera of reptilian coccidia. These four genera fall into two major phylogenetic groups including coccidian with tetra sporocystic dizoic oocysts known as the Eimeria-like coccidia and the
coccidia with disporocystic, tetrazoic exogenous oocysts in the genus *Isospora*. Importantly, my work provided the first genetic evidence for an undescribed genus of lizard coccidians with tetrasporocystic dizoic oocysts that do not fit the description of the two other tetrasporocystic dizoic oocyst containing coccidia including the genus *Acroeimeria* and the genus *Choleoeimeria*.

In the phylogenetic analysis for the *Eimeria*-like coccidia that have tetrasporocystic dizoic oocysts, the phylogenetic analyses revealed three distinct clades of *Eimeria*-like coccidia of lizards. One clade contains *Eimeria* i. s. *maboia* found in *H. mabouia* from Florida with intracytoplasmic development in the small intestine and defined as endogenous stages developing within the cytoplasm of intestinal epithelial cells that never expand into the intestinal lumen. This is unlike the two other clades which contain *Acroeimeria* and *Choleoeimeria*, that have epi cytoplasmic development in the small intestine or gall bladder, respectively. The next clade contains *Acroeimeria liner* found in *H. turcicus* from Oklahoma with epi cytoplasmic development in the small intestine where the intestinal epithelial cells containing endogenous stages enclosed in the microvillous border of the host cells expand into the intestinal lumen. The third clade contains *Choleoeimeria* *cf. turcicus* found in *H. mabouia* from Florida which has epi cytoplasmatic development where infected biliary epithelial cells that are hypertrophic expand into the lumen of the gall bladder (Paperna and Landsberg 1989b; Jirku et al. 2002). These phylogenetic analyses clearly indicate the evolutionary origin of multiple linages of *Eimeria*-like species infecting lizards.

In the phylogenetic analysis for Isosporan species from lizard hosts, my phylogenetic analyses also revealed that the 10 lizard *Isospora* species included in the analyses were distributed within three clades, although not all well supported. In this instance seven species of *Isospora* from lizard hosts formed a monophyletic clade. In contrast, the second clade includes six species of *Lankesterella* from amphibian, bird, and lizard hosts, and two *Isospora* and one *Caryospora* species from two species of geckos and the green anole, *Anolis carolinensis*,
respectively. In the last clade, *Isospora wiegmanniana* from the checkerboard worm lizard, *Trogonophis wiegmanni wiegmanni* (Megía-Palma et al., 2016) was positioned basally to the two clades mentioned above. These clades have interesting relationships both within their clade and with their sister clades. In the second clade, the oocysts of *Lankesterella* species never exit the vertebrate definitive host, but instead sporulate in the tissue of their definitive vertebrate host, which is unlike the other species in the clade (Barta et al., 2001). There is also an interesting relationship between the *Isospora* in that clade as only one of the clades contains *Isospora* species that all contain a polar granule in their oocysts. Clearly to evaluate these relationships and provide better statistical support for individual clades, future studies will need to increase the diversity of *Isopora* species within the analyses along with other lizard and reptilian coccidia including *Lankesterella* and *Caryospora* species.

In conclusion, to resolve these phylogenetic relationships more lizard species need to be sampled for their coccidian infection. Additionally, to evaluate these relationships from an evolutionary perspective we need to include a more diverse group of coccidians within our phylogenetic analyses including lizard coccidia that differ in their oocyst morphology (polar granules verses no polar granules) and endogenous development as mentioned above. Only by using the total evidence approach will be able to resolve the true evolutionary history of the coccidia. Finally, understanding these infections and their phylogenetic relationships will have important information that can be used to understand host parasite relationships which will become more important over the years as the effects of climate change and other human made dispersal events will modify existing ranges of lizards across the world.
REFERENCES


closely related to the genus *Lankesterella*: is the range of *Schellackia* restricted to the Old World? Parasites and Vectors 10: 470.


VITA

Allison Bryant

Candidate for the Degree of

Master of Science

Thesis: NEW INSIGHTS INTO REPTILIAN COCCIDAN INFECTIONS FROM TWO SPECIES OF INVASIVE GECKOS, THE MEDITERRANEAN HOUSE GECKO, HEMIDACTYLUS TURCICUS AND THE TROPICAL HOUSE GECKO, H. MABOUIA FROM THE NEW WORLD

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Rocky Mountain Conference of Parasitologists