ANEXOS

CERTIFICADO

Certificamos que a proposta intitulada "TAXONOMIA E ECOLOGIA DE COCCÍDIOS DE AVES SILVESTRES DO SUDESTE BRASILEIRO", protocolada sob o CEUA nº 6606250616, sob a responsabilidade de **Bruno Pereira Berto** *e equipe; Irlane Faria de Pinho; Lidiane Maria da Silva; Mariana Borges Rodrigues; Hermes Ribeiro Luz* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Instituto de Veterinária da Universidade Federal Rural do Rio de Janeiro (CEUA/UFRRJ) na reunião de 17/10/2016.

We certify that the proposal "TAXONOMY AND ECOLOGY OF COCCIDIA FROM WILD BIRDS FROM SOUTHEASTERN BRAZIL", utilizing 500 Birds (males and females), protocol number CEUA 6606250616, under the responsibility of **Bruno Pereira Berto** *and team; Irlane Faria de Pinho; Lidiane Maria da Silva; Mariana Borges Rodrigues; Hermes Ribeiro Luz* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Veterinary Institute of Rural Federal University of Rio de Janeiro (CEUA/UFRRJ) in the meeting of 10/17/2016.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 09/2016 a 08/2019 Área: Biologia Animal

Origem:	Não aplicável biotério						
Espécie:	Aves	sexo:	Machos e Fêmeas	idade:	1 a 240 meses	N:	500
Linhagem:	não se aplica			Peso:	10 a 10000 g		

Resumo: A Mata Atlântica é um dos biomas mais importantes para ser preservado na biodiversidade do planeta, sendo as regiões das baixadas do litoral do Sudeste brasileiro, onde está inserido o Estado do Rio de Janeiro, as mais prioritárias para conservação. A perda e fragmentação de habitats e a biopirataria são as principais ameaças, as quais, além dos impactos diretos a fauna, flora e microbiota, indiretamente favorecem a transmissão de parasitas e a susceptibilidade das aves. Neste contexto, surge a importância do conhecimento dos parasitas de aves silvestres, principalmente de alguns grupos pouco estudados, como os protozoários coccídios (Apicomplexa: Eucoccidiorida), os quais são de extrema importância, tanto em termos de biodiversidade, quanto em sua dinâmica e especificidade. Neste sentido, este projeto visa identificar e quantificar as espécies de coccídios de aves silvestres em áreas de Mata Atlântica do Estado do Rio de Janeiro, os quais fomentarão estudos complementares sobre a dinâmica do parasitismo entre famílias, hábitos, condições ambientais, etc. As expectativas são que os estudos dos oocistos revelem espécies novas, redescrições e novos hospedeiros, verificando a transmissão de coccídios entre aves de famílias distintas e possibilitando a elaboração de chaves dicotômicas de identificação. As distintas características ambientais e diferentes nichos ecológicos poderão influenciar na distribuição das espécies de coccídios, densidades e nas morfologia e morfometria dos oocistos. Desta forma, espécies distintas, padrões morfométricos e/ou morfológicos dos oocistos, e densidades serão associadas a cada condição ambiental, dado biométrico/biológico e nicho ecológico da ave hospedeira. Finalmente, as identificações e/ou elevadas densidades em determinada família, espécie ameaçada/endêmica ou aves em determinado ambiente poderão orientar ou priorizar a conservação de determinada ave e/ou localidade.

Local do experimento: Ambiente Silvestre

Seropédica, 17 de outubro de 2016

UFRRJ
Universidade Federal Rural do Rio de Janeiro

Comissão de Ética no Uso de Animais
Instituto de Veterinária

CEUA

Prof. Dr. Fabio Barbour Scott
Coordenador da Comissão de Ética no Uso de Animais
Instituto de Veterinária da Universidade Federal Rural do Rio de Janeiro

Prof. Dr. Jonimar Pereira Paiva
Vice-Coordenador da Comissão de Ética no Uso de Animais
Instituto de Veterinária da Universidade Federal Rural do Rio de Janeiro

CEUA

Comissão de Ética no Uso de Animais

Rio de Janeiro, 30 de agosto de 2019

DECLARAÇÃO DE APROVAÇÃO

Declaramos para os devidos fins que foi APROVADO o protocolo de número 021/2019 intitulado **"TAXONOMIA E ECOLOGIA DE COCCÍDIOS: IDENTIFICAÇÃO MORFOLÓGICA E MOLECULAR DE ESPÉCIES EM AVES SILVESTRES DO SUDESTE BRASILEIRO"**, encaminhado pelo pesquisador **Dr. Bruno Pereira Berto** do Departamento de Biologia Animal, Universidade Federal Rural do Rio de Janeiro (UFRRJ). Informamos que este parecer foi emitido em reunião ordinária da CEUA | UNIGRANRIO realizada no dia 28 de agosto de 2019, após avaliação do plenário da referida Comissão.

Vigência:	Setembro/2019 a Agosto/2022
Atividade:	Captura e coleta de amostras fecais
Número SISBIO:	42798-2
Grupo animal:	Aves silvestres (várias espécies)

DECLARATION OF APPROVAL

We hereby declare that protocol number 021/2019 entitled **"TAXONOMY AND ECOLOGY OF COCCIDIANS: MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF SPECIES IN WILD BIRDS IN SOUTHEASTERN BRAZIL"** has been APPROVED. This protocol was sent by **Dr. Bruno Pereira Berto**, Department of Animal Biology, Federal Rural University of Rio de Janeiro (UFRRJ). Please be informed that this opinion was delivered at the regular meeting of CEUA | UNIGRANRIO held on August 28, 2019, after evaluation by the plenary of that Committee.

Sergian Vianna Cardozo
Coordenador CEUA | UNIGRANRIO

Prof. Sergian V. Cardozo
Coordenador
Comissão de Ética no Uso de Animais
UNIGRANRIO

PROPEP UNIGRANRIO

UNIVERSIDADE UNIGRANRIO

Ministério do Meio Ambiente - MMA

Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio

Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 70132-6	Data da Emissão: 08/11/2021 18:11:24	Data da Revalidação*: 01/10/2022
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Bruno Pereira Berto	CPF: 103.532.617-50
Título do Projeto: TAXONOMIA E ECOLOGIA DE COCCÍDIOS: IDENTIFICAÇÃO MORFOLÓGICA E MOLECULAR DE ESPÉCIES EM AVES SILVESTRES DO SUDESTE BRASILEIRO	
Nome da Instituição: UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	CNPJ: 29.427.465/0001-05

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Publicação em periódicos e trabalhos de congressos	02/2020	09/2022
2	Levantamento bibliográfico	09/2019	09/2022
3	Estudo estatístico	09/2020	09/2022
4	Identificação morfológica e molecular	10/2019	09/2022
5	Captura de aves e coleta de amostras	09/2019	09/2022
6	Processamento das amostras	10/2019	09/2022

Equipe

#	Nome	Função	CPF	Nacionalidade
1	Mariana de Souza Oliveira	Bióloga (Doutoranda PPGBA)	152.850.237-00	Brasileira
2	Carlos Nei Ortúzar Ferreira	Bolsista IC (Discente de Graduação de Veterinária)	028.349.762-95	Brasileira
3	Jhon Lennon Genovez de Oliveira	Biólogo (Mestrando PPGBA)	142.851.307-85	Brasileira
4	Lucas de Assis Silva Andrade	Biólogo (Doutorando PPGBA)	147.520.527-92	Brasileira
5	Carla Maronezi	Veterinária	316.357.128-07	Brasileira
6	Sergian Vianna Cardozo	Veterinário (Professor UNIGRANRIO)	082.157.777-83	Brasileira
7	Ericson Ramos de Mello	Biólogo (Doutorando PPGCV)	113.246.467-64	Brasileira

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 70132-6	Data da Emissão: 08/11/2021 18:11:24	Data da Revalidação*: 01/10/2022
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Bruno Pereira Berto	CPF: 103.532.617-50
Título do Projeto: TAXONOMIA E ECOLOGIA DE COCCÍDIOS: IDENTIFICAÇÃO MORFOLÓGICA E MOLECULAR DE ESPÉCIES EM AVES SILVESTRES DO SUDESTE BRASILEIRO	
Nome da Instituição: UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	CNPJ: 29.427.465/0001-05

Observações e ressalvas

1	A autorização não exime o pesquisador da necessidade de obter outras anuências, como: I) do proprietário, arrendatário, posseiro ou morador quando as atividades forem realizadas em área de domínio privado ou dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso; II) da comunidade indígena envolvida, ouvido o órgão indigenista oficial, quando as atividades de pesquisa forem executadas em terra indígena; III) do Conselho de Defesa Nacional, quando as atividades de pesquisa forem executadas em área indispensável à segurança nacional; IV) da autoridade marítima, quando as atividades de pesquisa forem executadas em águas jurisdicionais brasileiras; V) do Departamento Nacional da Produção Mineral, quando a pesquisa visar a exploração de depósitos fossilíferos ou a extração de espécimes fósseis; VII do órgão gestor da unidade de conservação estadual, distrital ou municipal, dentre outras.
2	Este documento NÃO exime o pesquisador titular da necessidade de atender ao disposto na Instrução Normativa Ibama nº 27/2002, que regulamenta o Sistema Nacional de Anilhamento de Aves Silvestres.
3	Deve-se observar as as recomendações de prevenção contra à COVID-19 das autoridades sanitárias locais e das Unidades de Conservação a serem acessadas.
4	Esta autorização NÃO libera o uso da substância com potencial agrotóxico e/ou inseticida e NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de atender às exigências e obter as autorizações previstas em outros instrumentos legais relativos ao registro de agrotóxicos (Lei nº 7.802, de 11 de julho de 1989, Decreto nº 4.074, de 4 de janeiro de 2002, entre outros).
5	Esta autorização NÃO libera o uso da substância com potencial agrotóxico e/ou inseticida e NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de atender às exigências e obter as autorizações previstas em outros instrumentos legais relativos ao registro de agrotóxicos (Lei nº 7.802, de 11 de julho de 1989, Decreto nº 4.074, de 4 de janeiro de 2002, entre outros).
6	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
7	O titular de licença ou autorização e os membros da sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível, ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos, e empregar esforço de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condição in situ.
8	Este documento somente poderá ser utilizado para os fins previstos na Instrução Normativa ICMBio nº 03/2014 ou na Instrução Normativa ICMBio nº 10/2010, no que especifica esta Autorização, não podendo ser utilizado para fins comerciais, industriais ou esportivos. O material biológico coletado deverá ser utilizado para atividades científicas ou didáticas no âmbito do ensino superior.
9	Esta autorização NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena (FUNAI), da unidade de conservação estadual, distrital ou municipal, ou do proprietário, arrendatário, posseiro ou morador de área dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso.
10	Em caso de pesquisa em UNIDADE DE CONSERVAÇÃO, o pesquisador titular desta autorização deverá contactar a administração da unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infraestrutura da unidade.
11	O titular de autorização ou de licença permanente, assim como os membros de sua equipe, quando da violação da legislação vigente, ou quando da inadequação, omissão ou falsa descrição de informações relevantes que subsidiaram a expedição do ato, poderá, mediante decisão motivada, ter a autorização ou licença suspensa ou revogada pelo ICMBio, nos termos da legislação brasileira em vigor.

Este documento foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 70132-6	Data da Emissão: 08/11/2021 18:11:24	Data da Revalidação*: 01/10/2022
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Bruno Pereira Berto	CPF: 103.532.617-50
Título do Projeto: TAXONOMIA E ECOLOGIA DE COCCÍDIOS: IDENTIFICAÇÃO MORFOLÓGICA E MOLECULAR DE ESPÉCIES EM AVES SILVESTRES DO SUDESTE BRASILEIRO	
Nome da Instituição: UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	CNPJ: 29.427.465/0001-05

Observações e ressalvas

12	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/cgen

Outras ressalvas

1	1. Esta autorização não exime seu titular da necessidade de atender ao disposto na Instrução Normativa Ibama nº 27/2002, que regulamenta o Sistema Nacional de Anilhamento de Aves Silvestres. É obrigatório ao pesquisador portar autorização de anilhamento durante as atividades de campo que envolvam essa atividade. 2. O intervalo máximo de tempo para a vistoria de cada rede não pode ultrapassar 20 minutos, se a área for enxameada. Caso a área seja sem sombreada, os intervalos devem ser de 45 minutos, no máximo, de modo a evitar maior estresse aos animais. 3. O número máximo de redes armadas não deve ultrapassar 10 unidades para cada validador/anilhador presente.	CEMAVE Cabedelo-PB
2	- Comunicar ao PARNA S. Bocaina, com antecedência de 15 dias, quando serão feitas coletas/registros/amostragens de dados e informações no interior do Parque e em que localidade, e para solicitar apoio/alojamento, entrar em contato com o Parque com 03 semanas de antecedência. - Encaminhar listagem com a localização georreferenciada dos táxons ou dados coletados/registrados no PARNA S. Bocaina, indicando data das coletas/registros. - Encaminhar ao PARNA S. Bocaina cópia (física ou digital) das publicações decorrentes desta pesquisa, com endereço na rede mundial de computadores de onde podem ser encontradas. - Solicita-se a disponibilização de imagens registradas a fim de serem utilizadas em atividades do PARNA S. Bocaina, garantindo-se a indicação da autoria na veiculação. Todas as informações solicitadas e publicações resultantes da execução do projeto no interior do PARNA S. Bocaina deverão ser encaminhadas por meio eletrônico para: pesquisa.prbocaina@icmbio.gov.br	PARNA da Serra da Bocaina
3	- Em qualquer trilha utilizada durante a realização da pesquisa o pesquisador deverá fixar um a fita em uma árvore contendo o número da pesquisa no SISBIO. - Ao final da pesquisa o pesquisador deverá remover todas as fitas colocadas nas trilhas e outros objetos colocados em meio à floresta. - Todos os pontos, linhas e/ou caminhos de pesquisa deverão ser georreferenciados e os arquivos shapes deverão ser fornecidos à equipe da REBIO União, por email (rebiouniao@gmail.com). Neste caso, o pesquisador deverá fornecer o arquivo georreferenciado do local onde as câmeras serão colocadas, bem como as trilhas de acesso a elas. - Caso o pesquisador não identifique as trilhas utilizadas e forneça os arquivos shapes das áreas de pesquisas, a pesquisa poderá ser suspensa no SISBIO e as marcações ou objetos das pesquisas serem retirados da mata. - Em todos os trabalhos de campo na REBIO União a equipe de pesquisa deverá comunicar previamente à gestão da UC em quais locais estará durante a permanência na UC.	REBIO União

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 70132-6	Data da Emissão: 08/11/2021 18:11:24	Data da Revalidação*: 01/10/2022
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Bruno Pereira Berto	CPF: 103.532.617-50
Título do Projeto: TAXONOMIA E ECOLOGIA DE COCCÍDIOS: IDENTIFICAÇÃO MORFOLÓGICA E MOLECULAR DE ESPÉCIES EM AVES SILVESTRES DO SUDESTE BRASILEIRO	
Nome da Instituição: UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	CNPJ: 29.427.465/0001-05

Outras ressalvas

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 70132-6	Data da Emissão: 08/11/2021 18:11:24	Data da Revalidação*: 01/10/2022
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Bruno Pereira Berto	CPF: 103.532.617-50
Título do Projeto: TAXONOMIA E ECOLOGIA DE COCCÍDIOS: IDENTIFICAÇÃO MORFOLÓGICA E MOLECULAR DE ESPÉCIES EM AVES SILVESTRES DO SUDESTE BRASILEIRO	
Nome da Instituição: UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	CNPJ: 29.427.465/0001-05

4	"Ressalvas do PARQUE NACIONAL DA SERRA DOS ÓRGÃOS: COVID-19: O pesquisador somente poderá realizar atividade de campo após o término do estado de emergência devido à COVID-19, assim declarado por ato da autoridade competente. Recomendamos a leitura do Guia Elaborado pelo ICMBIO , Biodiversidade e COVID19 através do link: https://www.icmbio.gov.br/portal/images/stories/comunicacao/publicacoes/publicacoes-diversas/recomenadacoes_biodiversidade_e_covid19_ucs_e_outros_ambientes_naturais.pdf CONTATO PRÉVIO: dado o grande número de pesquisas em curso no Parque, a grande sobreposição espacial das mesmas e a potencial sobreposição nos focos de coleta da dados, sugerimos que a/o titular da autorização faça contato prévio <pesquisa.parnaso@icmbio.gov.br>, assim que recebê-la. Isso visa minimizar perda de tempo durante as expedições de campo e um melhor planejamento espacial da coleta de dados, evitando todas as possíveis interferências mútuas. AGENDAMENTO CAMPO / USO DE ESTRUTURAS: mensagem para < alojamento.icmbioteresopolis@icmbio.gov.br> com 15 dias antecedência. Informar nº autorização, total pessoas, datas (chegada e saída), necessidade ou não de alojamento, laboratório, salas etc. ATIVIDADES DIDÁTICAS: os locais de coleta devem ser informados para a Coordenação de Pesquisa do PARNASO de forma que não se sobreponham com outras pesquisas em andamento no Parque. Não está autorizada a coleta de espécimes da fauna e flora ameaçadas de extinção para atividades didáticas. ALTERAÇÕES EM CAMPO: devem ser discretas e no relatório final deve constar que "todo o material da pesquisa foi retirado?. REGISTROS DE ESPÉCIES DE INTERESSE PARA A CONSERVAÇÃO (ex: ameaçadas, novos táxons, interesse comercial, cinegético etc.) localizados em áreas de visitação devem ser informados à gestão do Parque para privilegiar sua proteção. PESSOAS E ATIVIDADES ESTRANHAS (ex: caçadores, visitantes fora da área adequada etc.): devem ser reportadas imediatamente à gestão do Parque (Chefia ou Coordenação de Pesquisa). UTILIZAÇÃO DE MÁQUINAS/MOTORES: devem ser feitos todos os esforços para minimizar a poluição visual e sonora. Não é permitido o uso de motores que derramem, mesmo que em quantidades pequenas, combustível ou óleo. PROCESSAMENTO/ARMAZENAMENTO DE MATERIAL: todo o material de coleta deve ser processado ou em campo ou no laboratório, jamais no alojamento, auditório ou refeitório! Materiais que exalem odores ou que obstruam passagem ou locais de assento devem ser retirados imediatamente e não podem ser armazenados também do lado de fora das estruturas por conta do acesso de animais nativos que circulam (ex: quatis, primatas, serpentes, artrópodes).

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 70132-6	Data da Emissão: 08/11/2021 18:11:24	Data da Revalidação*: 01/10/2022
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Bruno Pereira Berto	CPF: 103.532.617-50
Título do Projeto: TAXONOMIA E ECOLOGIA DE COCCÍDIOS: IDENTIFICAÇÃO MORFOLÓGICA E MOLECULAR DE ESPÉCIES EM AVES SILVESTRES DO SUDESTE BRASILEIRO	
Nome da Instituição: UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	CNPJ: 29.427.465/0001-05

ANIMAIS CAPTURADOS/COLETADOS: O manejo de animais vivos ou mortos devem ser evitados em áreas de visitação/turismo. Só deve ser feito em campo (área sem visitação) ou no laboratório de apoio do Parque.

PLANTAS COLETADAS: duplicatas devem ser depositadas no JBRJ e no Herbário do PARNASO (conforme Plano de Manejo, mas recomenda-se antes consultar Coordenação de Pesquisa do Parque).

DEFESA PÚBLICAS: solicitamos que todas as defesas públicas (graduação, especialização, mestrado e doutorado, além de apresentações em eventos) relacionadas a esta autorização sejam, quando possível, comunicadas à gestão do Parque para que gestores possam conhecimento dos debates acerca dos dados que estão sendo produzidos pelas pesquisas feitas no PARNASO. Pedimos que as mensagens sejam enviadas para: pesquisa.parnaso@icmbio.gov.br, parnaso@icmbio.gov.br"

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 70132-6	Data da Emissão: 08/11/2021 18:11:24	Data da Revalidação*: 01/10/2022

De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.

Dados do titular

Nome: Bruno Pereira Berto	CPF: 103.532.617-50
Título do Projeto: TAXONOMIA E ECOLOGIA DE COCCÍDIOS: IDENTIFICAÇÃO MORFOLÓGICA E MOLECULAR DE ESPÉCIES EM AVES SILVESTRES DO SUDESTE BRASILEIRO	
Nome da Instituição: UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	CNPJ: 29.427.465/0001-05

Locais onde as atividades de campo serão executadas

#	Descrição do local	Município-UF	Bioma	Caverna?	Tipo
1	Ilha da Marambaia	Mangaratiba-RJ	Mata Atlântica	Não	Fora de UC Federal
2	Condomínio Parque das Águas	Guapimirim-RJ	Mata Atlântica	Não	Fora de UC Federal
3	Fragmentos de Mata Atlântica no distrito de Cacaria	Piraí-RJ	Mata Atlântica	Não	Fora de UC Federal
4	Área de Relevante Interesse Ecológico Floresta da Cicuta	Volta Redonda-RJ	Mata Atlântica	Não	Dentro de UC Estadual
5	RPPN Reserva Porangaba	Itaguaí-RJ	Mata Atlântica	Não	Dentro de UC Estadual
6	Parque Nacional da Serra da Bocaina	RJ	Mata Atlântica	Não	Dentro de UC Federal
7	Campus IFRJ Pinheiral	Pinheiral-RJ	Mata Atlântica	Não	Fora de UC Federal
8	Fragmentos de Mata Atlântica no distrito de Santa Rita de Cássia	Barra Mansa-RJ	Mata Atlântica	Não	Fora de UC Federal
9	Floresta Nacional Mario Xavier	Seropédica-RJ	Mata Atlântica	Não	Dentro de UC Estadual
10	Campus UFRRJ Seropédica	Rio de Janeiro-RJ	Mata Atlântica	Não	Fora de UC Federal
11	Parque Nacional da Serra dos Órgãos	RJ	Mata Atlântica	Não	Dentro de UC Federal
12	Parque Nacional do Itatiaia	RJ	Mata Atlântica	Não	Dentro de UC Federal

Atividades

#	Atividade	Grupo de Atividade
1	Coleta/transporte de amostras biológicas in situ	Fora de UC Federal
2	Coleta/transporte de amostras biológicas in situ	Dentro de UC Federal
3	Captura de animais silvestres in situ	Fora de UC Federal
4	Captura de animais silvestres in situ	Dentro de UC Federal
5	Marcação de animais silvestres in situ	Fora de UC Federal
6	Marcação de animais silvestres in situ	Dentro de UC Federal

Atividades X Táxons

#	Atividade	Táxon	Qtde.
1	Marcação de animais silvestres in situ	Aves	-
2	Coleta/transporte de amostras biológicas in situ	Aves	-
3	Captura de animais silvestres in situ	Aves	-

A quantidade prevista só é obrigatória para atividades do tipo "Coleta/transporte de espécimes da fauna silvestre in situ". Essa quantidade abrange uma porção territorial mínima, que pode ser uma Unidade de Conservação Federal ou um Município.

Este documento foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

Código de autenticação: 0701320620211108

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 70132-6	Data da Emissão: 08/11/2021 18:11:24	Data da Revalidação*: 01/10/2022
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Bruno Pereira Berto	CPF: 103.532.617-50
Título do Projeto: TAXONOMIA E ECOLOGIA DE COCCÍDIOS: IDENTIFICAÇÃO MORFOLÓGICA E MOLECULAR DE ESPÉCIES EM AVES SILVESTRES DO SUDESTE BRASILEIRO	
Nome da Instituição: UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	CNPJ: 29.427.465/0001-05

A quantidade significa: por espécie X localidade X ano.

Materiais e Métodos

#	Tipo de Método (Grupo taxonômico)	Materiais
1	Amostras biológicas (Aves)	Ectoparasita, Fezes, Penas
2	Método de captura/coleta (Aves)	Rede de neblina
3	Método de marcação (Aves)	Anilha de Alumínio (padrão CEMAVE), Anilha metálica (padrão CEMAVE)

Destino do material biológico coletado

#	Nome local destino	Tipo destino
1	UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	Laboratório

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 70132-6	Data da Emissão: 08/11/2021 18:11:24	Data da Revalidação*: 01/10/2022
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Bruno Pereira Berto	CPF: 103.532.617-50
Título do Projeto: TAXONOMIA E ECOLOGIA DE COCCÍDIOS: IDENTIFICAÇÃO MORFOLÓGICA E MOLECULAR DE ESPÉCIES EM AVES SILVESTRES DO SUDESTE BRASILEIRO	
Nome da Instituição: UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	CNPJ: 29.427.465/0001-05

Registro de coleta imprevista de material biológico

De acordo com a Instrução Normativa nº03/2014, a coleta imprevista de material biológico ou de substrato não contemplado na autorização ou na licença permanente deverá ser anotada na mesma, em campo específico, por ocasião da coleta, devendo esta coleta imprevista ser comunicada por meio do relatório de atividades. O transporte do material biológico ou do substrato deverá ser acompanhado da autorização ou da licença permanente com a devida anotação. O material biológico coletado de forma imprevista, deverá ser destinado à instituição científica e, depositado, preferencialmente, em coleção biológica científica registrada no Cadastro Nacional de Coleções Biológicas (CCBIO).

Táxon*	Qtde.	Tipo de Amostra	Qtde.	Data

* Identificar o espécime do nível taxonômico possível.

Este documento foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

Código de autenticação: 0701320620211108

Ministério do Meio Ambiente - MMA

Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio

Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 54951-3	Data da Emissão: 20/09/2018 12:43:30	Data da Revalidação*: 20/09/2019
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Bruno Pereira Berto	CPF: 103.532.617-50
Nome da Instituição: UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	CNPJ: 29.427.465/0001-05

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Processamento das amostras	08/2016	07/2019
2	Estudo estatistico	10/2016	07/2019
3	Captura de aves e coleta de amostras	07/2016	06/2019
4	Publicação em periódicos e trabalhos de congressos	11/2016	07/2019
5	Identificação dos coccídios	09/2016	07/2019

Observações e ressalvas

1	A autorização não eximirá o pesquisador da necessidade de obter outras anuências, como: I) do proprietário, arrendatário, posseiro ou morador quando as atividades forem realizadas em área de domínio privado ou dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso; II) da comunidade indígena envolvida, ouvido o órgão indigenista oficial, quando as atividades de pesquisa forem executadas em terra indígena; III) do Conselho de Defesa Nacional, quando as atividades de pesquisa forem executadas em área indispensável à segurança nacional; IV) da autoridade marítima, quando as atividades de pesquisa forem executadas em águas jurisdicionais brasileiras; V) do Departamento Nacional da Produção Mineral, quando a pesquisa visar a exploração de depósitos fossilíferos ou a extração de espécimes fósseis; VI) do órgão gestor da unidade de conservação estadual, distrital ou municipal, dentre outras.
2	O titular de autorização ou de licença permanente, assim como os membros de sua equipe, quando da violação da legislação vigente, ou quando da inadequação, omissão ou falsa descrição de informações relevantes que subsidiaram a expedição do ato, poderá, mediante decisão motivada, ter a autorização ou licença suspensa ou revogada pelo ICMBio, nos termos da legislação brasileira em vigor.
3	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
4	O titular de licença ou autorização e os membros da sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível, ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos; e empregar esforço de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condição in situ.
5	Esta autorização NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena (FUNAI), da unidade de conservação estadual, distrital ou municipal, ou do proprietário, arrendatário, posseiro ou morador de área dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso.
6	Este documento somente poderá ser utilizado para os fins previstos na Instrução Normativa ICMBio n° 03/2014 ou na Instrução Normativa ICMBio n° 10/2010, no que especifica esta Autorização, não podendo ser utilizado para fins comerciais, industriais ou esportivos. O material biológico coletado deverá ser utilizado para atividades científicas ou didáticas no âmbito do ensino superior.
7	Em caso de pesquisa em UNIDADE DE CONSERVAÇÃO, o pesquisador titular desta autorização deverá contactar a administração da unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infra-estrutura da unidade.
8	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/cgen.

Este documento foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

Código de autenticação: 0549510320180920

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 54951-3	Data da Emissão: 20/09/2018 12:43:30	Data da Revalidação*: 20/09/2019
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Bruno Pereira Berto	CPF: 103.532.617-50
Nome da Instituição: UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	CNPJ: 29.427.465/0001-05

Locais onde as atividades de campo serão executadas

#	Descrição do local	Município-UF	Bioma	Caverna?	Tipo
1	Parque Nacional do Itatiaia	RJ	Mata Atlântica	Não	Dentro de UC Federal

Atividades X Táxons

#	Atividade	Táxon	Qtde.
1	Captura de animais silvestres in situ	Aves	-
2	Coleta/transporte de amostras biológicas in situ	Aves	-

Materiais e Métodos

#	Tipo de Método (Grupo taxonômico)	Materiais
1	Amostras biológicas (Aves)	Fezes
2	Método de captura/coleta (Aves)	Rede de neblina

Destino do material biológico coletado

#	Nome local destino	Tipo destino
1	UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	Coleção

Ministério do Meio Ambiente - MMA

Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio

Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 54951-3	Data da Emissão: 20/09/2018 12:43:30	Data da Revalidação*: 20/09/2019
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Bruno Pereira Berto	CPF: 103.532.617-50
Nome da Instituição: UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	CNPJ: 29.427.465/0001-05

Registro de coleta imprevista de material biológico

De acordo com a Instrução Normativa nº03/2014, a coleta imprevista de material biológico ou de substrato não contemplado na autorização ou na licença permanente deverá ser anotada na mesma, em campo específico, por ocasião da coleta, devendo esta coleta imprevista ser comunicada por meio do relatório de atividades. O transporte do material biológico ou do substrato deverá ser acompanhado da autorização ou da licença permanente com a devida anotação. O material biológico coletado de forma imprevista, deverá ser destinado à instituição científica e, depositado, preferencialmente, em coleção biológica científica registrada no Cadastro Nacional de Coleções Biológicas (CCBIO).

Táxon*	Qtde.	Tipo de Amostra	Qtde.	Data

* Identificar o espécime do nível taxonômico possível.

Este documento foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

Código de autenticação: 0549510320180920

Ministério do Meio Ambiente - MMA

Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio

Sistema de Autorização e Informação em Biodiversidade - SISBIO

Licença permanente para coleta de material zoológico

Número: 42798-4	Data da Emissão: 29/06/2020 17:35:50	Data da Revalidação*: 01/04/2021
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Bruno Pereira Berto	CPF: 103.532.617-50
Nome da Instituição: UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	CNPJ: 29.427.465/0001-05

Observações e ressalvas

1	A autorização não eximirá o pesquisador da necessidade de obter outras anuências, como: I) do proprietário, arrendatário, posseiro ou morador quando as atividades forem realizadas em área de domínio privado ou dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso; II) da comunidade indígena envolvida, ouvido o órgão indigenista oficial, quando as atividades de pesquisa forem executadas em terra indígena; III) do Conselho de Defesa Nacional, quando as atividades de pesquisa forem executadas em área indispensável à segurança nacional; IV) da autoridade marítima, quando as atividades de pesquisa forem executadas em águas jurisdicionais brasileiras; V) do Departamento Nacional da Produção Mineral, quando a pesquisa visar a exploração de depósitos fossilíferos ou a extração de espécimes fósseis; VI) do órgão gestor da unidade de conservação estadual, distrital ou municipal, dentre outras.
2	O pesquisador somente poderá realizar atividade de campo após o término do estado de emergência devido à COVID-19, assim declarado por ato da autoridade competente.
3	A licença permanente não é válida para: a) coleta ou transporte de espécies que constem nas listas oficiais de espécies ameaçadas de extinção; b) manutenção de espécimes de fauna silvestre em cativeiro; c) recebimento ou envio de material biológico ao exterior; e d) realização de pesquisa em unidade de conservação federal ou em caverna. A restrição prevista no item d não se aplica às categorias Reserva Particular do Patrimônio Natural e Área de Proteção Ambiental constituídas por terras privadas.
4	Esta licença permanente não poderá ser utilizada para fins comerciais, industriais ou esportivos ou para realização de atividades integrantes do processo de licenciamento ambiental de empreendimentos.
5	Esta licença permanente NÃO exime o pesquisador titular da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena (FUNAI), da unidade de conservação estadual, distrital ou municipal.
6	O titular de autorização ou de licença permanente, assim como os membros de sua equipe, quando da violação da legislação vigente, ou quando da inadequação, omissão ou falsa descrição de informações relevantes que subsidiaram a expedição do ato, poderá, mediante decisão motivada, ter a autorização ou licença suspensa ou revogada pelo ICMBio, nos termos da legislação brasileira em vigor.
7	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
8	A licença permanente será válida enquanto durar o vínculo empregatício do pesquisador com a instituição científica a qual ele estava vinculado por ocasião da solicitação.
9	O titular de licença ou autorização e os membros da sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível, ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos; e empregar esforço de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condição in situ.
10	O pesquisador titular da licença permanente, quando acompanhado, deverá registrar a expedição de campo no Sisbio e informar o nome e CPF dos membros da sua equipe, bem como dados da expedição, que constarão no comprovante de registro de expedição para eventual apresentação à fiscalização.
11	O titular da licença permanente deverá apresentar, anualmente, relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias após o aniversário de emissão da licença permanente.
12	O pesquisador titular da licença permanente será responsável pelos atos dos membros da equipe (quando for o caso)
13	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/cgen.
14	Este documento NÃO exime o pesquisador titular da necessidade de atender ao disposto na Instrução Normativa Ibama nº 27/2002, que regulamenta o Sistema Nacional de Anilhamento de Aves Silvestres.

Este documento foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

Código de autenticação: 0427980420200629

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Licença permanente para coleta de material zoológico

Número: 42798-4	Data da Emissão: 29/06/2020 17:35:50	Data da Revalidação*: 01/04/2021
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Bruno Pereira Berto	CPF: 103.532.617-50
Nome da Instituição: UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	CNPJ: 29.427.465/0001-05

Outras ressalvas

1	No caso do uso simultâneo de mais de 10 redes de neblina, o pesquisador deverá estar acompanhado de ao menos uma pessoa experiente na manipulação de aves, a fim de conseguir revisar todas as redes em intervalos menores que 30 minutos.	CEMAVE Cabedelo-PB

Atividades

#	Atividade	Grupo de Atividade
1	Coleta/transporte de espécimes da fauna silvestre in situ	Fora de UC Federal
2	Coleta/transporte de amostras biológicas in situ	Fora de UC Federal
3	Captura de animais silvestres in situ	Fora de UC Federal
4	Marcação de animais silvestres in situ	Fora de UC Federal

Táxons autorizados

#	Nível taxonômico	Táxon(s)
1	Classe	Animalia > Chordata > Aves

Destino do material biológico coletado

#	Nome local destino	Tipo destino
1	UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	Coleção

Ministério do Meio Ambiente - MMA

Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio

Sistema de Autorização e Informação em Biodiversidade - SISBIO

Licença permanente para coleta de material zoológico

Número: 42798-4	Data da Emissão: 29/06/2020 17:35:50	Data da Revalidação*: 01/04/2021
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Bruno Pereira Berto	CPF: 103.532.617-50
Nome da Instituição: UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO	CNPJ: 29.427.465/0001-05

Registro de coleta imprevista de material biológico

De acordo com a Instrução Normativa nº03/2014, a coleta imprevista de material biológico ou de substrato não contemplado na autorização ou na licença permanente deverá ser anotada na mesma, em campo específico, por ocasião da coleta, devendo esta coleta imprevista ser comunicada por meio do relatório de atividades. O transporte do material biológico ou do substrato deverá ser acompanhado da autorização ou da licença permanente com a devida anotação. O material biológico coletado de forma imprevista, deverá ser destinado à instituição científica e, depositado, preferencialmente, em coleção biológica científica registrada no Cadastro Nacional de Coleções Biológicas (CCBIO).

Táxon*	Qtde.	Tipo de Amostra	Qtde.	Data

* Identificar o espécime do nível taxonômico possível.

Este documento foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

Código de autenticação: 0427980420200629

Isospora oliveirai *n. sp. (Chromista: Miozoa: Eimeriidae) from the Greenish Schiffornis* Schiffornis virescens *(Lafresnaye, 1838) (Passeriformes: Tyranni: Tityridae) in South America*

Carlos Nei Ortúzar-Ferreira, Jhon Lennon Genovez-Oliveira, Mariana de Souza Oliveira, et al.

Acta Parasitologica

ISSN 1230-2821
Volume 65
Number 4

Acta Parasit. (2020) 65:843-851
DOI 10.1007/s11686-020-00237-8

Springer

Acta Parasitologica (2020) 65:843–851
https://doi.org/10.1007/s11686-020-00237-8

ORIGINAL PAPER

Isospora oliveirai n. sp. (Chromista: Miozoa: Eimeriidae) from the Greenish Schiffornis *Schiffornis virescens* (Lafresnaye, 1838) (Passeriformes: Tyranni: Tityridae) in South America

Carlos Nei Ortúzar-Ferreira[1] · Jhon Lennon Genovez-Oliveira[2] · Mariana de Souza Oliveira[2] · Ericson Ramos de Mello[3] · Sergio Thode-Filho[4] · Águida Aparecida de Oliveira[5] · Viviane Moreira de Lima[6] · Ildemar Ferreira[6] · Bruno Pereira Berto[6]

Received: 24 April 2020 / Accepted: 27 May 2020 / Published online: 3 June 2020

Abstract

Background Coccidia are obligatory intracellular parasites with at least one intestinal phase in their life cycles, being *Isospora* Schneider, 1881 the main coccidian genus related to the order Passeriformes. However, there is no record of isosporans from the passerine family Tityridae, which is the family of the greenish schiffornis *Schiffornis virescens* (Lafresnaye, 1838).

Purpose This study aimed to examine the faeces from a greenish schiffornis *S. virescens* captured in the Itatiaia National Park, State of Rio de Janeiro, Southeastern Brazil, to determine what coccidian parasites were present.

Methods Only one specimen of *Schiffornis virescens* was captured with mist nets. Coccidian oocysts were recovered from the fecal samples by flotation in Sheather's saturated solution. Morphological observations, line drawings, photomicrographs and measurements were made in optical microscopy and digitally edited. The molecular analysis included the study of the sequence of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene, with phylogenetic reconstructions based on the Neighbor-Joining and Maximum Likelihood analysis.

Results An *Isospora* sp. considered as new to science is described and identified from *Schiffornis virescens* (Lafresnaye, 1838). *Isospora oliveirai* n. sp. has oocysts that are subspheroidal, 26.0×24.8 µm, with rough, bilayered wall, *c.*2.5 µm thick. Micropyle and oocyst residuum absent, but one to six polar granules are present. Sporocysts lemon-shaped, 18.1×10.9 µm. The Stieda body is knob-like to half-moon-shaped and sub-Stieda is rounded. Sporocyst residuum is present, composed of scattered spherules of different sizes. Sporozoites are vermiform, with refractile bodies and nucleus. Molecular analysis at the COI gene exhibited similarity of 97% with *Isospora serinuse* Yang, Brice, Elliot et Ryan, 2015 from island canaries *Serinus canaria* (Linnaeus, 1758), and *Isospora* spp. from great tits *Parus major* (Linnaeus, 1758) and European robins *Erithacus rubecula* (Linnaeus, 1758).

Conclusion Based on the morphological and molecular features, *I. oliveirai* is considered as new to science and the first coccidian species recorded from Tityridae.

Keywords Morphology · Molecular biology · Taxonomy · Phylogeny · Coccidia · Oocysts · Neotropical birds · Suboscines · Tityridae · Parque Nacional do Itatiaia

✉ Bruno Pereira Berto
berto.ufrrj@gmail.com

[1] Curso de Graduação em Medicina Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil

[2] Programa de Pós-graduação em Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil

[3] Programa de Pós-graduação em Ciências Veterinárias, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil

[4] Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro, Duque de Caxias, Brazil

[5] Departamento de Microbiologia e Imunologia Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil

[6] Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, BR 465, Km 7, Seropédica, Rio de Janeiro CEP 23890-000, Brazil

Introduction

Coccidia is a subclass of the Infraphylum Apicomplexa, which is characterized as obligatory intracellular parasites with at least one intestinal phase in their life cycles [1, 2]. They have worldwide distribution and a biological cycle consisting of merogony, gametogony and sporogony [3]. Among many genera, *Isospora* Schneider, 1881 (Apicomplexa: Eucoccidiorida) is a coccidian genus of the family Eimeriidae that is widely found in birds, especially in the order Passeriformes [4, 5].

The greenish schiffornis *Schiffornis virescens* (Lafresnaye, 1838) is a passerine bird belonging to the family Tityridae. It is endemic to the Atlantic Forest inhabiting alone or in pairs the understory stratum, where it feeds on fruits and insects. It is also a species sensitive to forest fragmentation with low to medium abundance in its habitats [6, 7]. Besides Brazil, this species also is distributed in Argentina and Paraguay [8].

The coccidian parasitism in wild birds is widely reported in the scientific literature [4]; although clinical signs are hardly observed when these hosts are in their natural habitats [9, 10]. This context is widely observed in the Itatiaia National Park (Parque Nacional do Itatiaia), which is a protected area with a high degree of vulnerability, located in the Serra da Mantiqueira, Southeastern Brazil [11]. Birds in the Itatiaia National Park have often been reported to be parasitized by coccidians in the last years, but always without perceptible clinical signs of coccidiosis [12–15].

The current study aimed to examine the faeces from a greenish schiffornis *S. virescens* captured in the Itatiaia National Park (Parque Nacional do Itatiaia), a conservation unit in Southeastern Brazil, to determine what coccidian parasites were present.

Materials and Methods

Sample Collection

In May 2017 an expedition was conducted in the Itatiaia National Park (22°27′20″S, 44°36′28″W) to capture wild birds with mist nets and collect fecal samples. Among the captured birds, there was only one specimen of *S. virescens*. This greenish schiffornis was kept in an individual box and faeces collected immediately after defecation. After identification of the species, the bird was photographed and released and stool samples were placed in centrifuge tubes containing a potassium dichromate 2.5% ($K_2Cr_2O_7$) solution at 1:6 (v/v).

Morphological Analyses

Samples were taken to the Laboratório de Biologia de Coccídios, Universidade Federal Rural do Rio de Janeiro (UFRRJ). Samples were incubated at room temperature (25 °C) for 10 days or until ~70% of the oocysts were sporulated. Oocysts were isolated by flotation in Sheather's sugar saturated solution (specific gravity: 1.20) and examined microscopically using the technique described by Duszynski and Wilber [16] and Berto et al. [17]. Morphological observations, line drawings, photomicrographs and measurements were made using an Olympus BX binocular microscope (Olympus Optical, Tokyo, Japan) coupled to a digital camera Eurekam 5.0 (BEL Photonics, Monza, Italy). Line drawings were edited using two software applications from CorelDRAW® (Corel Draw Graphics Suite, Version 11.0, Corel Corporation, Canada), i.e., Corel DRAW and Corel PHOTO-PAINT. All measurements are in micrometres and are given as the range followed by the mean in parentheses.

Molecular Analyses

Sixteen oocysts carefully identified with the same characteristic features under light microscopy were isolated and resuspended in PBS [18]. DNA was extracted from the oocyst using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, São Paulo, Brazil) according to the manufacturer's instructions. To fully lyse the oocyst, four freeze–thaw cycles were applied prior to the DNA extraction. The PCR amplification for the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene was carried out using a nested PCR, as previously described by Dolnik et al. [18] and Yang et al. [19]. The external primers COIbF1 (5′-GWT CAT TAG TAT GGG CAC ATC A-3′) and COIbR1 (5′-CCA AGA GAT AAT ACR AAR TGG AA-3′) produced a PCR product of c.302 bp in size. The internal primes COIbF2 (5′-GGG CAC ATC ATA TGA TGA C-3′) and COIbR2 (5′-ATA GTA TGT ATC ATG TAR WGC AA-3′) produced an amplicon of 257 bp in size. The PCR reaction contained 10 µl of 5× Green GoTaq® Flexi Buffer, 3 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTPs, 0.4 µM of each primer, 1.25 units of GoTaq® DNA polymerase, 3 µl of DNA (for primary reaction) or 3 µl primary PCR product (for the secondary reaction). Both primary and secondary PCR were conducted using the same cycling conditions: 1 cycle of 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 47 °C for 45 s, and 72 °C for 1 min and a final extension of 72 °C for 5 min. The amplicons from the second round of PCR were purified using the Qiagen MinElute PCR Purification (Qiagen, São Paulo, Brazil). All PCR products were sequenced using the PCR forward and reverse primers by Ludwig Biotechnology, were an ABI-Prism 3500 Genetic Analyzer (Applied Biosystems,

Foster City, California) was used for Sanger sequencing. The results of the sequencing reactions were analyzed and edited using the program Chromas 2.6.

DNA Sequence Analyses

The newly generated sequences were compared to those for *Isospora* spp. and other coccidian parasites available on the GenBank database using the Basic Local Alignment Search Tool (BLAST). Phylogenetic trees were constructed for *Isospora* spp. at the COI sequences for additional isolates from GenBank. Alignment and parsimony analyses were conducted using MEGA version 7 [20]. The evolutionary history was inferred using the Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods and the distances were computed using the Tamura-Nei method based on model selection using ModelTest in MEGA 7. Bootstrap analyses were conducted using 1,000 replicates to assess the reliability of inferred tree topologies.

Results

The captured greenish schiffornis *S. virescens* was positive for coccidia. All observed oocysts were characteristic of *Isospora*. This material is described below.

Family Eimeriidae Minchin, 1903

Genus *Isospora* Schneider, 1881

***Isospora oliveirai* Ortúzar-Ferreira et Berto n. sp.** (Figs. 1, 2)

Oocysts ($n = 26$) subspheroidal, 24–28 × 23–27 (26.0 × 24.8); length/width (*L*/*W*) ratio 1.0–1.1 (1.05). Wall bilayered, 2.3–2.7 (2.5) thick, outer layer slightly rough, c.2/3 of total thickness. Micropyle and oocyst residuum absent, but one to six polar granules are present, appearing to be membrane-bounded when more than three are present. Sporocysts ($n = 26$) lemon-shaped, 17–19 × 10–11 (18.1 × 10.9); *L*/*W* ratio 1.6–1.7 (1.65). Stieda body present, knob-like to half-moon-shaped, 2.0 × 3.5; sub-Stieda body present, rounded, 2.5 × 3.5; para-Stieda body absent; sporocyst residuum present, composed of scattered spherules of different sizes. Sporozoites vermiform, with anterior and posterior refractile bodies and centrally located nucleus.

Type host: *Schiffornis virescens* (Lafresnaye, 1838) (Aves: Passeriformes: Tyranni: Tityridae), greenish schiffornis.

Type locality: Parque Nacional do Itatiaia (22°27′20″S, 44°36′28″W), southeastern Brazil.

Type specimens: Photosyntypes, line drawing, and oocysts in 70% ethanol are deposited at the Museu de Zoologia at the Universidade Federal Rural do Rio de Janeiro, Brazil, under the accession number MZURMTZ2020022. Phototypes and line drawings are also deposited and available (http://r1.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under the repository number P-105/2020. Photographs of the type-host specimen (symbiotype) are deposited in the same collection.

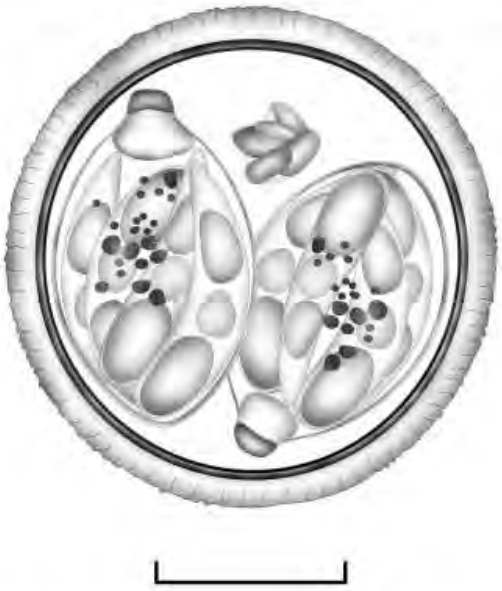

Fig. 1 Composite line drawing of the sporulated oocyst of *Isospora oliveirai* n. sp. from the greenish schiffornis *Schiffornis virescens*. Scale-bar 10 µm

Site in host: Unknown.

Representative DNA sequence: One representative COI sequence was deposited in the GenBank database under the accession number MT276845.

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature [21] details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Isospora oliveirai* is urn:lsid:zoobank.org:act:46CE4322-1798-477C-A4E2-B2B66FD15279.

Etymology: The specific name is derived from the family name of the Brazilian parasitologist Dr Francisco Carlos Rodrigues de Oliveira, given in his honor for his contribution to the study of helminth and protozoan parasites.

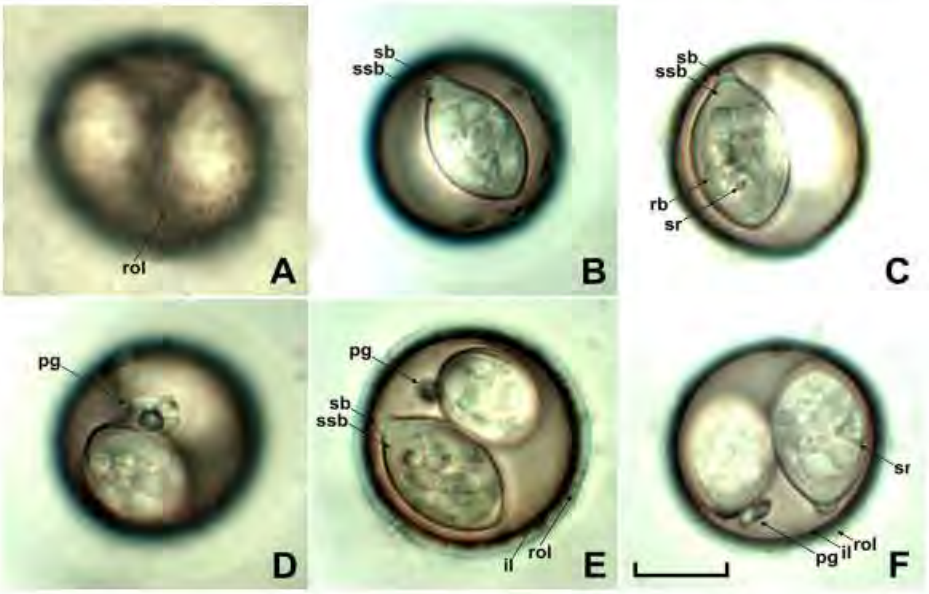

Fig. 2 Photomicrographs of sporulated oocysts of *Isospora oliveirai* n. sp. from the greenish schiffornis *Schiffornis virescens*. *il* inner and *ol* rough outer layers of the oocyst wall, *pg* polar granule, *sb* Stieda and *ssb* sub-Stieda bodies, *sr* sporocyst residuum, *rb* refractile body. All to same scale. Scale-bar 10 µm

Remarks: The morphology of *Isospora* spp. recorded from the parvorder Tyrannida and of *I. oliveirai* recovered from *S. virescens* in the current study are shown in Table 1. *Isospora araponga* Doležalová, Torres, Fernández et Modrý, 2004 is differentiated from *I. oliveirai,* because it has a smooth oocyst wall, asymmetric sporocysts and Stieda and sub-Stieda bodies barely discernible; while *I. oliveirai* has a rough wall, lemon-shaped symmetric sporocysts, and prominent Stieda and sub-Stieda bodies. *Isospora lopesi* Silva-Carvalho et Berto, 2018 can also be easily differentiated by its smooth oocyst wall, unique polar granule and flattened Stieda body; which are different in *I. oliveirai* (Table 1). The other coccidian species recorded of tyranids can be differentiated from *I. oliveirai*, among other aspects, also by the smooth oocyst wall, presence of up to two polar granules, and differences in the shape of the Stieda and Sub-Stieda bodies. Finally, *I. oliveirai* differs from all these coccidian species, because it has the largest oocysts described, to date, in the parvorder Tyrannida (Table 1).

Phylogenetic analysis: DNA amplification of the oocyst of *I. oliveirai* showed a clear band of c.250 bp. Phylogenetic analysis included 23 sequences for avian *Isospora* spp. available on GenBank (Fig. 3). *Eimeria tenella* (Railliet et Lucet, 1891) was used as the outgroup. *Isospora oliveirai* sat separately on the cladogram and had the highest similarity of 97% with *Isospora serinuse* Yang, Brice, Elliot et Ryan, 2015 from island canaries *Serinus canaria* (Linnaeus, 1758), and *Isospora* spp. from great tits *Parus major* Linnaeus, 1758 and European robins *Erithacus rubecula* (Linnaeus, 1758) [19, 22].

Discussion

As evidenced in studies on taxonomy of coccidians, there is a specificity at the family level in coccidian parasitism within the order Passeriformes [4, 5, 16]. Thus, the taxonomic description of a new species requires a morphological comparison with the coccidian species recorded from the same family of the host; however, there is no description of coccidians in the family Tityridae, where *S. virescens* belongs. In this sense, the coccidian oocysts from the current study were compared with the species described at higher taxonomic levels of the host.

The parvorder Tyrannida brings together the family Pipridae and the superfamilies Cotingoidea (Oxyruncidae, Onychorhynchidae, Tityridae and Cotingidae) and Tyrannoidea (Pipritidae, Platyrinchidae, Tachurisidae, Rhynchocyclidae and Tyrannidae) [23]. Only four of these ten families of Tyrannida has coccidian species recorded: (1) *I. araponga* in Cotingidae; (2) *Isospora feroxis* Berto, Luz, Flausino, Ferreira et Lopes, 2009 and *Isospora attilae* Rodrigues, Silva, Lopes, Berto, Luz, Ferreira et Lopes, 2015 in Tyrannidae; (3) *Isospora mionectesi* Berto, Flausino, Luz, Ferreira et Lopes, 2009 in Rhynchocyclidae and (4) *I. lopesi* in Platyrinchidae [24–28]. As shown in Table 1, *I. oliveirai* is easily differentiated from these coccidian species by several characteristic features, such as: roughness of the outer layer of the oocyst wall, number and arrangement of polar granules, shape of the Stieda and sub-Stieda bodies, in addition to that the oocysts of *I. oliveirai* are larger than all species recorded from the parvorder Tyrannida.

Table 1 Comparative morphology of *Isospora* spp. recorded from the parvorder Tyrannida

Coccidia	Hosts	References	Oocysts						Sporocysts						
			Shape	Length (µm)	Width (µm)	*L/W* ratio	Wall	Polar granule	Shape	Length (µm)	Width (µm)	*L/W* ratio	Stieda body	Substieda body	Residuum
Isospora araponga Doležalová, Torres, Fernández et Modrý, 2004	*Procnias nudicollis* (Vieillot, 1817) (Cotingoidea: Cotingidae)	Dolezalová et al. [24]	Subspheroidal to ellipsoidal	17–22 (19.5)	14–16 (15.5)	1.1–1.4 (1.3)	Smooth (1.0)	Present, 1–3	Ellipsoidal slightly asymmetric	12–13 (12.5)	7–9 (8.5)	1.3–1.7 (1.5)	Barely visible	Absent	Compact
Isospora feroxis Berto, Luz, Flausino, Ferreira et Lopes, 2009	*Myiarchus ferox* (Gmelin, 1789) (Tyrannoidea: Tyrannidae)	Berto et al. [26]	Subspheroidal	18–20 (18.7)	17–20 (18)	1.0–1.1 (1.1)	Smooth (1.2)	Present, usually 2	Ovoidal	11–13 (11.7)	8–10 (8.5)	1.0–1.5 (1.4)	Flattened	Prominent	Diffuse
Isospora mionectesi Berto, Flausino, Luz, Ferreira et Lopes, 2009	*Mionectes rufiventris* Cabanis, 1846 (Tyrannoidea: Rhynchocyclidae)	Berto et al. [25]	Ellipsoidal	26–31 (28.3)	19–23 (21.2)	1.2–1.4 (1.3)	Smooth (1.3)	Present, 1 or 2	Elongate ellipsoidal	17–22 (19.7)	10–13 (11.7)	1.6–1.8 (1.7)	Rounded	Prominent	Compact
Isospora attilae Rodrigues, Silva, Lopes, Berto, Luz, Ferreira et Lopes, 2015	*Attila rufus* (Vieillot, 1819) (Tyrannoidea: Tyrannidae)	Rodrigues et al. [27]	Subspheroidal to ellipsoidal	18–22 (20.3)	18–21 (19)	1.1–1.2 (1.07)	Smooth (1.3)	Present, 1 or 2	Ellipsoidal	12–15 (13.5)	7–9 (7.9)	1.6–1.9 (1.7)	Knob-like	Rounded to trapezoidal	Diffuse

Table 1 (continued)

Coccidia	Hosts	References	Oocysts						Sporocysts						
			Shape	Length (µm)	Width (µm)	*L*/*W* ratio	Wall	Polar granule	Shape	Length (µm)	Width (µm)	*L*/*W* ratio	Stieda body	Substieda body	Residuum
Isospora lopesi Silva-Carvalho et Berto, 2018	*Platyrinchus mystaceus* Vieillot, 1818 (Tyrannoidea: Platyrinchidae)	Silva-Carvalho et al. [28]	Subspheroidal to ovoidal	18–24 (20.6)	18–22 (19.7)	1.0–1.2 (1.05)	Smooth (1.5)	Present, single	Ellipsoidal	12–16 (14.4)	8–11 (8.6)	1.5–1.9 (1.7)	Flattened to half-moon-shaped	Rounded	Diffuse
Isospora oliveirai Ortúzar-Ferreira et Berto n. sp.	*Schiffornis virescens* (Lafresnaye, 1838) (Cotingoidea: Tityridae)	Current work	Subspheroidal	24–28 (26.0)	23–27 (24.8)	1.0–1.1 (1.05)	Slightly rough (2.5)	Present, 1–6	Lemon-shaped	10–11 (10.5)	6–7 (6.5)	1.6–1.7 (1.6)	Knob-like to half-moon-shaped	Rounded	Diffuse

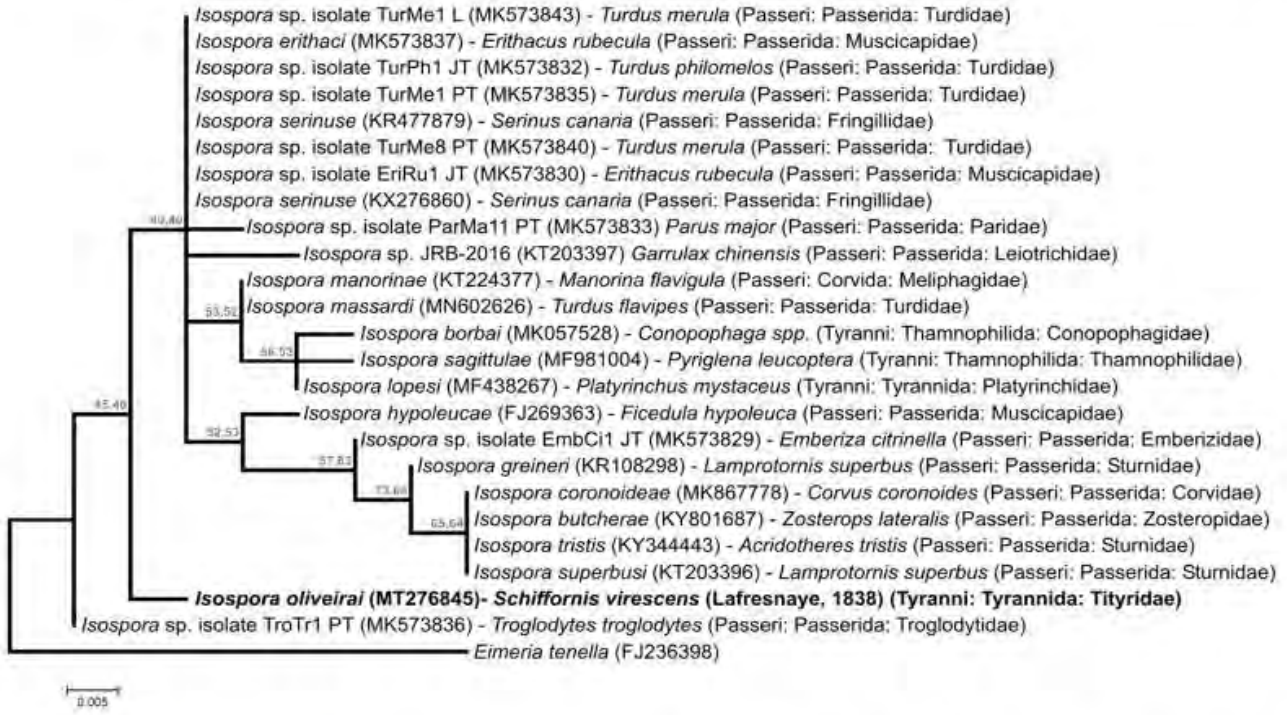

Fig. 3 Maximum likelihood tree estimated from the COI sequences. Numbers at nodes represent bootstrap support (1000 replicates; only values > 40% shown) for neighbor-joining and maximum likelihood, respectively. The scale-bar represents the number of nucleotide substitutions per site

The rough oocyst wall observed in *I. oliveirai* is an uncommon morphological feature in *Isospora* spp.; therefore, it becomes the most distinguishable characteristic of this species. This reasoning can be exemplified outside the parvorder Tyrannida: *Isospora machadoae* Pinho, Silva, Rodrigues, Lopes, Oliveira, Luz, Ferreira, Lopes et Berto, 2018 recorded from the family Turdidae and *Isospora borbai* Silva-Carvalho et Berto, 2019, recorded from the family Conopophagidae are the only species within their host-families to have a rough oocyst wall, and, therefore, are easily differentiated from the others species [14, 15].

Phylogenetic analysis did not place *I. oliveirai* in any monophyly; on the contrary, it was separated from a large clade containing several *Isospora* spp. from Passeriformes (Fig. 3). This result can be explained by the close similarities between these *Isospora* spp. in this large clade, which vary between 98 and 100%, whereas *I. oliveirai* differed from these *Isospora* spp. by more than 3%. Anyway, the main inconsistency in phylogeny was in the comparison with *I. lopesi*, which is the only to be classified in the same parvorder (Tyrannida) as *I. oliveirai* among the coccidian species that has COI sequences deposited in Genbank; however, it had a low similarity with *I. oliveirai* of only 95% and sit a distant clade with other coccidian species reported from the Suborder Tyranni (suboscines). Thus, these results are not conclusive in establishing the phylogeny of *I. oliveirai* and other *Isospora* spp. from Passeriformes; however, these should be more elucidative as more *Isospora* spp. are sequenced for the COI gene and deposited in the Genbank.

Finally, based on the morphological and molecular features described above, *I. oliveirai* is considered as new to science and the first coccidian species recorded from Tityridae.

Acknowledgements We are thankful to staff at the Parque Nacional do Itatiaia, mainly to the research coordinator Dr. Léo Nascimento, that allowed us to access and use some facilities during the expeditions.

Author Contributions The study was designed by VML, IF and BPB. Field work was performed by CNO-F, JLG-O, MSO, ST-F and BPB. Laboratory procedures for maintenance, recovery, measurements, photomicrographs and isolation of oocysts were performed by CNO-F and MSO. DNA extraction, amplification and sequencing were performed by CNO-F, JLG-O, ERM, AAO and VML. BPB analyzed the data and drew the coccidian oocyst. The manuscript was written by CNO-F and BPB and subsequently revised by all other authors.

Funding This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ). CNO-F has a scholarship from CNPq (Grant/Award number 120107/2018-0). JLG-O and MSO have scholarship from CAPES (Grant/Award number 001). BPB has a fellowship from CNPq (Grant/

Award number 303899/2019-0) and from FAPERJ (Grant/Award number E-26/202.797/2019).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval Field-collecting permits were issued by SISBIO/ICMBio (license 54951-1) and CEUA/IV/UFRRJ (protocol 6606250616). All applicable institutional, national and international guidelines for the care and use of animals were followed.

References

1. Fayer R (1980) Epidemiology of protozoan infection the coccidia. Vet Parasitol 6:75–103. https://doi.org/10.1016/0304-4017(80)90039-4
2. Ruggiero MA, Gordon DP, Orrell TM, Bailly N, Bourgoin T, Brusca RC, Cavalier-Smith T, Guiry MD, Kirk PM (2015) Correction: a higher level classification of all living organisms. PLoS ONE 10:e0130114. https://doi.org/10.1371/journal.pone.0119248
3. Duszynski DW, Couch L, Upton SJ (2000) The coccidia of the World. https://www.k-state.edu/parasitology/worldcoccidia. Accessed 01 Feb 2020
4. Berto BP, Flausino W, McIntosh D, Teixeira-Filho WL, Lopes CWG (2011) Coccidia of New World passerine birds (Aves: Passeriformes): a review of *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae). Syst Parasitol 80:159–204. https://doi.org/10.1007/s11230-011-9317-8
5. Berto BP, Lopes CWG (2013) Distribution and dispersion of Coccidia in wild passerines of the Americas. In: Ruiz L, Iglesias L (eds) Birds: evolution and behavior, breeding strategies, migration and spread of disease. Nova Science Publishers, New York, pp 47–66
6. Stotz DF, Fitzpatrick JW, Parker TA III, Moskovits DK (1996) Aves neotropicais: ecologia e conservação. Universidade de Chicago Press, Chicago
7. Anjos L, Collins CD, Holt RD, Volpato GH, Mendonça LB, Lopes EV, Boçon R, Bisheimer MV, Serafini PP, Carvalho J (2011) Padrões de ocupação de abundância de espécies de aves e sensibilidade à fragmentação florestal: implicações para conservação no brasileiro Mata Atlântica. Biol Conserv 144:2213–2222. https://doi.org/10.1016/j.biocon.2011.05.013
8. Sigrist T (2014) Guia de Campo: Avifauna Brasileira. Avis Brasilis, São Paulo
9. Massey JG (2003) Diseases and management of wild Passeriformes. Semin Avian Exot Pet Med 12:29–36. https://doi.org/10.1053/saep.2003.127876
10. Atkinson CT, Thomas NJ, Hunter DB (2008) Parasitic diseases of wild birds. Wiley-Blackwell, Singapore
11. ICMBIO (2018) Parque Nacional do Itatiaia. http://www.icmbio.gov.br/parnaitatiaia. Accessed 23 Feb 2020
12. Silva LM, Rodrigues MB, Lopes BB, Berto BP, Luz HR, Ferreira I, Lopes CWG (2016) A new coccidian, *Isospora parnaitatiaiensis* n. sp. (Apicomplexa, Eimeriidae), from the white-shouldered fire-eye *Pyriglena leucoptera* (Passeriformes, Thamnophilidae) from South America. Parasitol Res 115:745–749. https://doi.org/10.1007/s00436-015-4798-z
13. Pinho IF, Rodrigues MB, Silva LM, Lopes BB, Oliveira MS, Ferreira MA, Cardozo SV, Luz HR, Ferreira I, Lopes CWG, Berto BP (2017) Characterization and distribution of *Isospora sabiai* n. sp. (Apicomplexa: Eimeriidae) from thrushes *Turdus* spp. (Passeriformes: Turdidae) from Brazil. J Parasitol 103:285–29.. https://doi.org/10.1645/16-152
14. Pinho IF, Silva LM, Rodrigues MB, Lopes BB, Oliveira, Luz HR, Ferreira I, Lopes CWG, Berto BP (2018) *Isospora machadoae* sp. nov. (Protozoa: Apicomplexa: Eimeriidae), a new coccidian species from white-necked thrushes *Turdus albicolis* (Passeriformes: Turdidae) of South America. Zoologia 35:e24570. https://doi.org/10.3897/zoologia.35.e24570
15. Silva-Carvalho LM, Oliveira JLG, Oliveira MS, Rodrigues MB, Abreu ST, Ortúzar-Ferreira CN, Franco HA, Thode-Filho S, Oliveira AA, Lima VM, Ferreira I, Berto BP (2019) *Isospora borbai* n. sp. (Chromista: Apicomplexa: Eimeriidae) from gnateaters *Conopophaga* spp. (Passeriformes: Tyranni: Conopophagidae) in South America. Acta Parasitol 64:6117–6624. https://doi.org/10.2478/s11686-019-00079-z
16. Duszynski D, Wilber P (1997) A guideline for the preparation of species descriptions in the Eimeriidae. J Parasitol 83:333–336. https://doi.org/10.2307/3284470
17. Berto BP, McIntosh D, Lopes CWG (2014) Studies on coccidian oocysts (Apicomplexa: Eucoccidiorida). Rev Bras Parasitol Vet 23:1–15. https://doi.org/10.1590/S1984-29612014001
18. Dolnik OV, Palinauskas V, Bensch S (2009) Individual cocysts of *Isospora* (Apicomplexa: Coccidia) parasites from avian faeces: from photo to sequence. J Parasitol 95:169–174. https://doi.org/10.1645/GE-1873.1
19. Yang R, Brice B, Elliot A, Ryan U (2015) *Isospora serinuse* n. sp. (Apicomplexa: Eimeriidae) from a domestic canary (*Serinus canaria* forma domestica) (Passeriformes: Fringillidae) in Western Australia. Exp Parasitol 159:59–66. https://doi.org/10.1016/j.exppara.2015.08.020
20. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599. https://doi.org/10.1093/molbev/msm092
21. ICZN (2012) International Commission on Zoological Nomenclature: amendment of articles 8, 9, 10, 21 and 78 of the International Code of Zoological Nomenclature to expand and refine methods of publication. Bull Zool Nomencl 69:161–169. https://doi.org/10.21805/bzn.v69i3.a8.161
22. Trefancová A, Kvičerová J (2019) *Isospora svecica* sp. n. (Apicomplexa: Eimeriidae), a new species of coccidium from the white-spotted bluethroat *Luscinia svecica cyanecula* (Aves: Passeriformes: Muscicapidae). Parasitol Res 118:3043–3051. https://doi.org/10.1007/s00436-019-06397-5
23. Piacentini VQ, Aleixo A, Agne CE, Maurício GN, Pacheco JF, Bravo GA, Brito GRR, Naka LN, Olmos F, Posso S, Silveira LF, Betini GS, Carrano E, Franz I, Lees AC, Lima LM, Pioli D, Schunck F, Amaral FR, Bencke GA, Cohn-Haft M, Figueiredo LFA, Straube FC, Cesari E (2015) Annotated checklist of the birds of Brazil by the Brazilian Ornithological Records Committee/Lista comentada das aves do Brasil pelo Comitê Brasileiro de Registros Ornitológicos. Rev Bras Ornitol 23:90–298
24. Dolezalová M, Torres J, Fernández H, Modrý D (2004) *Isospora araponga* sp. n. (Apicomplexa: Eimeriidae), a new species of Isospora Schneider from a bare-throated bellbird, *Procnias nudicollis* (Vieillot, 1817) (Passeriformes: Cotingidae) from Brazil. Mem Inst Oswaldo Cruz 99:829–830. https://doi.org/10.1590/S0074-02762004000800008
25. Berto BP, Flausino W, Luz HR, Ferreira I, Lopes CWG (2009) *Isospora mionectesi* sp. nov. (Apicomplexa, Eimeriidae) from the grey-hooded flycatcher, *Mionectes rufiventris* in Brazil. Acta Parasitol 54:301–304. https://doi.org/10.2478/s11686-009-0053-z
26. Berto BP, Luz HR, Flausino W, Ferreira I, Lopes CWG (2009) New species of *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) from the short-crested flycatcher

Myiarchus ferox (Gmelin) (Passeriformes: Tyrannidae) in South America. Syst Parasitol 74:75–80. https://doi.org/10.1007/s11230-009-9197-3

27. Rodrigues MB, Silva LM, Lopes BDB, Berto BP, Luz HR, Ferreira I, Lopes CWG (2015) A new species of *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) from the grey-hooded attila *Attila rufus* Vieillot, 1819 (Passeriformes: Tyrannidae) on the Marambaia Island, Brazil. Zootaxa 4034:193–196. https://doi.org/10.11646/zootaxa.4034.1.10
28. Silva-Carvalho LM, Pastura DGN, Gomes JV, Siqueira PB, Rodrigues MB, Lima VM, Berto BP (2018) *Isospora lopesi* n. sp. (Protozoa: Apicomplexa: Eimeriidae) from the eastern white-throated spadebill *Platyrinchus mystaceus* Vieillot (Passeriformes: Tyranni: Tyrannidae) in South America. Syst Parasitol 95:455–463. https://doi.org/10.1007/s11230-018-9795-z

Syst Parasitol (2021) 98:333–341
https://doi.org/10.1007/s11230-021-09981-5

Redescription and molecular identification of *Isospora feroxis* Berto, Luz, Flausino, Ferreira & Lopes, 2009 (Eimeriidae) from tyrant-flycatchers (Tyrannoidea) in South America

Carlos Nei Ortúzar-Ferreira · Ericson Ramos de Mello · Jennifer Oliveira Melo · Mariana de Souza Oliveira · Sergio Thode-Filho · Sergian Vianna Cardozo · Águida Aparecida de Oliveira · Viviane Moreira de Lima · Ildemar Ferreira · Bruno Pereira Berto

Received: 5 June 2020 / Accepted: 23 April 2021 / Published online: 6 May 2021

Abstract In the present study *Isospora feroxis* Berto, Luz, Flausino, Ferreira & Lopes, 2009 is redescribed from the photosyntypes and from new samples from a short-crested flycatcher *Myiarchus ferox* (Gmelin), which is the type-host in the type-locality, the Marambaia Island in Southeastern Brazil. In addition, the yellow-olive flycatcher *Tolmomyias sulphurescens* Spix is recorded as a new host for this species, in a new locality, the Itatiaia National Park, in the interior of Southeastern Brazil, providing a preliminary genotypic characterization via sequencing of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene. Micropyle and rough oöcyst wall are added to the description of *I. feroxis*, in addition to other details. This is the sixth species identified from suboscine birds (Tyranni) to have a COI gene sequence deposited in GenBank and, although it is not yet possible to make conclusions on the phylogeny

C. N. Ortúzar-Ferreira · J. O. Melo
Curso de Graduação em Medicina Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7,
23897-000 Seropédica, Rio de Janeiro, Brazil

E. R. de Mello
Programa de Pós-Graduação em Ciências Veterinárias, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7,
23897-000 Seropédica, Rio de Janeiro, Brazil

M. de Souza Oliveira
Programa de Pós-Graduação em Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7,
23897-000 Seropédica, Rio de Janeiro, Brazil

S. Thode-Filho
Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro, Av. República do Paraguai 120,
25050-100 Duque de Caxias, Brazil

S. V. Cardozo
Programa de Pós-Graduação em Biomedicina Translacional, Universidade do Grande Rio, Rua Professor José de Souza Herdy 1160,
25071-202 Duque de Caxias, RJ, Brazil

Á. A. de Oliveira
Departamento de Microbiologia e Imunologia Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7,
23897-000 Seropédica, Rio de Janeiro, Brazil

V. M. de Lima · I. Ferreira · B. P. Berto (✉)
Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7,
23897-000 Seropédica, Rio de Janeiro, Brazil
e-mail: berto.ufrrj@gmail.com

of *Isospora* spp. from Passeriformes by the COI gene, the molecular analysis confirmed the differences between coccidian species from tyrant-flycatchers.

Introduction

Biodiversity in the Neotropical Region is very relevant for researchers around the world, among various aspects, due to its potential and recurring new discoveries (Tundisi & Matsumura-Tundisi, 2008). In this context, the class Aves stands out for its great diversity and exuberance, attracting not only scientists, but also ecotourists, especially birdwatchers (Stotz et al., 1996). Brazil is the second country in the Neotropical region with the largest number of bird species; where, currently, there are 1919 species listed by the Ornithological Records Committee of Brazil (Piacentini et al., 2015). In the context of research on Neotropical birds, there is the study of their parasites, which has been increasingly related to ecology, physiology and conservation of wild species. Among bird parasites, coccidian protozoa stand out as the cause of morbidity and mortality in unfavorable conditions of host and environment, to a commensalism-like in bird populations in conserved and balanced environments (Berto & Lopes, 2020).

Tyrant-flycatchers represent a superfamily, Tyrannoidea (Piacentini et al., 2015), or simply the family Tyrannidae, according to BirdLife International (Del Hoyo & Collar, 2016), which distribution extends across North, Central and South America, being more concentrated in the Neotropical Region. It is currently the largest superfamily/family of the class Aves worldwide, with 450 species recorded. In Brazil, 218 species are listed (Piacentini et al., 2015; Del Hoyo & Collar, 2016). Even with this great diversity, there are few reports of coccidian species from tyrant-flycatchers, when compared to other bird families. In this context, the current study aimed to redescribe *Isospora feroxis* Berto, Luz, Flausino, Ferreira & Lopes, 2009 from the photosyntypes and from samples of a short-crested flycatcher *Myiarchus ferox* (Gmelin), which is the type-host in the type-locality, the Marambaia Island in Southeastern Brazil. In addition, the yellow-olive flycatcher *Tolmomyias sulphurescens* Spix is recorded as a new host for this species, in a new locality, the Itatiaia National Park, in the interior of Southeastern Brazil, providing a preliminary genotypic characterization via sequencing of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene.

Materials and methods

Sample collection

Between August 2014 and August 2018, 19 expeditions were conducted in different locations in the Itatiaia National Park (22°27′S, 44°36′W), a protected area with a high degree of vulnerability, located in the Serra da Mantiqueira on the border of the States of Rio de Janeiro, Minas Gerais and São Paulo (ICMBIO, 2016), to capture wild birds with mist nets and collect faecal samples. A total of seven yellow-olive flycatchers *T. sulphurescens* were captured. In addition to the Itatiaia National Park, an expedition in September 2014 was conducted on Maramabia Island (23°3′38.86"S, 43°58′47.56"W), on the coast of the state of Rio de Janeiro, Southeastern Brazil, where one short-crested flycatcher *M. ferox* was captured. The birds were kept in individual boxes and faeces collected immediately after defecation. After identification of the species, the bird was photographed and released and faecal samples were placed in centrifuge tubes containing a potassium dichromate 2.5% ($K_2Cr_2O_7$) solution at 1:6 (v/v).

Morphological analyses

Samples were taken to the Laboratório de Biologia de Coccídios, Universidade Federal Rural do Rio de Janeiro (UFRRJ). Samples were incubated at room temperature (25°C) for 10 days or until ~70% of the oöcysts were sporulated. Oöcysts were isolated by flotation in Sheather's sugar saturated solution (specific gravity: 1.20) and examined microscopically using the technique described by Duszynski & Wilber (1997) and Berto et al. (2014). Morphological observations, line drawings, photomicrographs and measurements were made using an Olympus BX binocular microscope (Olympus Optical, Tokyo, Japan) coupled to a digital camera Eurekam 5.0 (BEL Photonics, Monza, Italy). Line drawings were edited using Corel DRAW® and Corel PHOTO-PAINT (Corel Draw Graphics Suite, Version 11.0, Corel Corporation, Canada). All measurements are in micrometres and

are given as the range followed by the mean in parentheses.

Molecular analyses

An individual oöcyst from a fecal sample of *T. sulphurescens* was isolated from serial dilutions of the oöcysts in drops on a microscope slide using a sterile micropipette. This isolated oöcyst was resuspended in PBS and washed by centrifuging until the supernatant became clear (Dolnik et al., 2009). DNA was extracted from the purified oöcysts using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, São Paulo, Brazil) according to the manufacturer's instructions. In order to fully lyse the oöcyst, four freeze-thaw cycles were applied prior to the DNA extraction. The PCR amplification for the COI gene was carried out using a nested PCR, as previously described by Dolnik et al. (2009) and Yang et al. (2015). The external primers COIbF1 (5′-GWT CAT TAG TAT GGG CAC ATC A-3′) and COIbR1 (5′-CCA AGA GAT AAT ACR AAR TGG AA-3′) produced a PCR product of 302 bp in size. The internal primes COIbF2 (5′-GGG CAC ATC ATA TGA TGA C-3′) and COIbR2 (5′-ATA GTA TGT ATC ATG TAR WGC AA-3′) produced an amplicon of 257 bp in size. The PCR reaction contained 10 µl of 5× Green GoTaq® Flexi Buffer, 3 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTPs, 0.4 µM of each primer, 1.25 units of GoTaq® DNA polymerase, 3 µl of DNA (for primary reaction) or 3µl primary PCR product (for the secondary reaction). Both primary and secondary PCR were conducted using the same cycling conditions: 1 cycle of 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 47°C for 45 s, and 72°C for 1 min and a final extension of 72°C for 5 min. The amplicons from the second round of PCR were purified using the Qiagen MinElute PCR Purification (Qiagen, São Paulo, Brazil). All PCR products were sequenced using the PCR forward and reverse primers by Ludwig Biotechnology, were an ABI-Prism 3500 Genetic Analyzer (Applied Biosystems, Foster City, California) was used for Sanger sequencing. The results of the sequencing reactions were analyzed and edited using the program Chromas 2.6 (Technelysium Pty Ltd, Queensland Australia).

DNA sequence analyses

The newly generated sequence was compared to those for *Isospora* spp. and other coccidian parasites available on the GenBank database using the Basic Local Alignment Search Tool (BLAST). Phylogenetic trees were constructed for *Isospora* spp. at the COI sequences for additional isolates from GenBank. Alignment and parsimony analyses were conducted using MEGA version 7, using ClustalW as alignment algorithm (Tamura et al., 2007). The evolutionary history was inferred using the Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods and the distances were computed using the Tamura-Nei method based on model selection using ModelTest in MEGA 7. Bootstrap analyses were conducted using 1,000 replicates to assess the reliability of inferred tree topologies.

Obtaining the photosyntypes of Isospora feroxis *Berto, Luz, Flausino, Ferreira & Lopes, 2009*

The photosyntypes of *I. feroxis* from *M. ferox* identified in Berto et al. (2009a), which were deposited in the Parasitology Collection of the Laboratório de Biologia de Coccídios (http://r1.ufrrj.br/labicoc/colecao.html) at UFRRJ under repository number P-30/2009, were required for morphological comparison.

Results

Seven *T. sulphurescens* were examined and five of them (71%), which were captured in the same location in the Itatiaia National Park, known as "Trilha das Borboletas" or "Butterfly Trail" (22°26′57.00"S, 44°36′25.00"W), were positive for coccidia. The short-crested flycatcher *M. ferox* captured in Maramabia Island was also positive for coccidia. All observed oöcysts were morphologically identified as *I. feroxis*. This material is described below.

Eimeriidae Minchin, 1903

***Isospora* Schneider, 1881**

***Isospora feroxis* Berto, Luz, Flausino, Ferreira & Lopes, 2009**

Type-host: *Myiarchus ferox* (Gmelin) (Aves: Passeriformes: Tyranni: Tyrannoidea: Tyrannidae) short-crested flycatcher.
Other host: Tolmomyias sulphurescens Spix (Aves: Passeriformes: Tyranni: Tyrannoidea: Rhynchocyclidae) yellow-olive flycatcher (present study).
Type-locality: Marambaia Island (23°3′38.86"S, 43°58′47.56"W), Southeastern Brazil
Other locality: Parque Nacional do Itatiaia - Itatiaia National Park (22°26′57.00"S, 44°36′25.00"W), Southeastern Brazil.
Type-specimens: Photosyntypes and line drawing are deposited and available (http://r1.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under the repository number P-30/2009.
Other specimens (present study): Photomicrographs, line drawing, and oöcysts in 2.5% $K_2Cr_2O_7$ solution (Williams et al., 2010) from *T. sulphurescens* are deposited at the Museu de Zoologia at the Universidade Federal Rural do Rio de Janeiro, Brazil, under accession number MZURPTZ2020025. Photomicrographs are also deposited and available (http://r1.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under repository numbers 108/2020 (*T. sulphurescens*) and 109/2020 (*M. ferox*). Photovouchers of the host specimens are deposited in the same collection.
Site in host: Unknown; oöcysts recovered from faeces.
Prevalence: 75% (6/8) overall; 71% (5/7) for *T. sulphurescens*; and 100% (1/1) for *M. ferox*.
Representative DNA sequence: Representative COI sequences of the oöcysts from *T. sulphurescens* were deposited in the GenBank database under the accession number MT563402.

Description (Figs. 1, 2)
Sporulated oöcyst

Oöcysts (n = 81) subspheroidal, 18–23 × 18–23 (20.7 × 20.0); length/width (L/W) ratio 1.0-1.1 (1.04). Wall bi-layered, 1.3-2.0 (1.7) thick, outer layer with minimal to moderate roughness, *c*.2/3 of total thickness. Micropyle present, 4.4-9.2 (7.4) wide. Oöcyst residuum absent, but 1-3 (usually 2 bonded) polar granules are present.
Sporocyst and sporozoites
Sporocysts (n = 70) 2, ovoidal to ellipsoidal, 11–15 × 8–10 (13.4 × 9.2); L/W ratio 1.3-1.6 (1.45). Stieda body present, flattened to half-moon-shaped, 0.5–0.7 × 1.3–2.2 (0.6 × 1.7); substieda body present, rounded to trapezoidal, 1.0–1.8 × 2.4–3.3 (1.3 × 2.9), infrequently with prominence resembling a compartmentalized substieda; parastieda body absent; sporocyst residuum present, composed of spherules of different sizes. Sporozoites 4, vermiform, 10–11 × 3–4 (10.9 × 3.6), with posterior refractile body and centrally located nucleus.

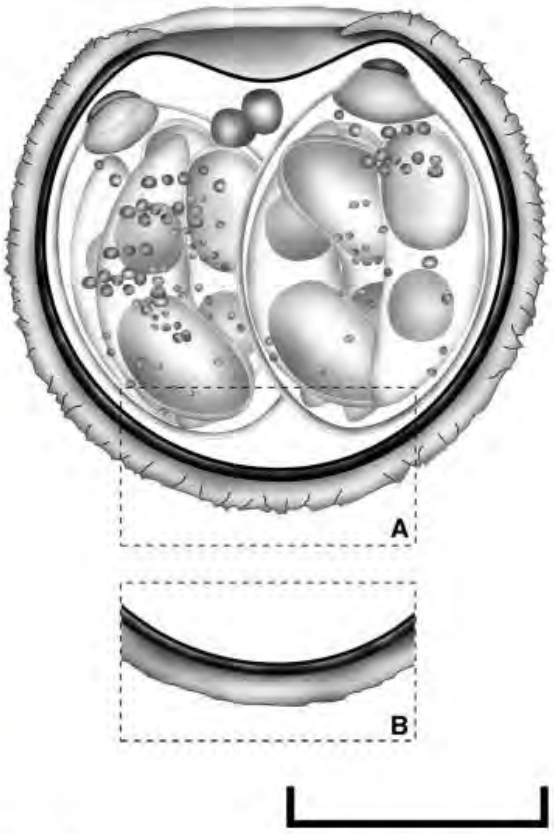

Fig. 1 Composite line drawing of the sporulated oöcyst for redescription of *Isospora feroxis* from tyrant-flycatchers, highlighting the outer layer of the oöcyst wall with moderate (A) or minimal (B) roughness. Scale-bar: 10 µm.

Remarks
Four *Isospora* spp. are recorded from New World tyrant-flycatchers (Table 1). To date, *I. feroxis* had the smallest oöcysts among these *Isospora* spp.; however, in the present study, the wide addition of oöcyst measurements from two hosts increased the range of oöcysts measurements, making them compatible with *Isospora attilae* Rodrigues, Silva, Lopes, Berto, Luz, Ferreira & Lopes, 2015 and *Isospora lopesi* Silva-Carvalho & Berto, 2018. However, *I. feroxis* oöcysts

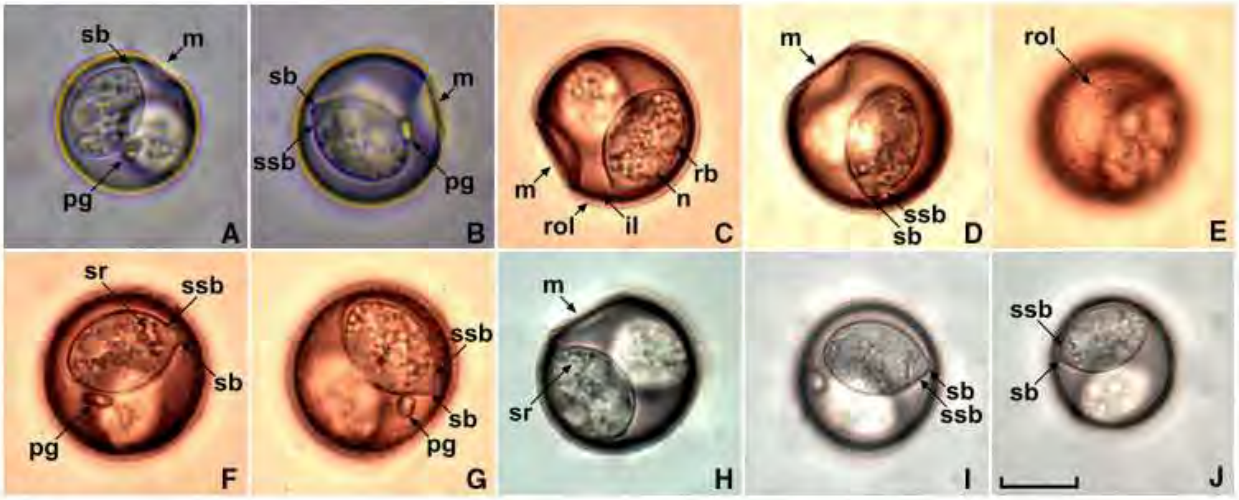

Fig. 2 Sporulated oöcysts of *Isospora feroxis* from the photosyntypes (A, B) and from new samples of a short-crested flycatcher *Myiarchus ferox* (C–G) and of yellow-olive flycatchers *Tolmomyias sulphurescens* (H–J). Note the inner (il) and rough outer (rol) layers of the oöcyst wall; micropyle (m); nucleus (n); polar granule (pg); Stieda (sb) and sub-Stieda bodies (ssb); sporocyst residuum (sr); refractile body (rb). Scale-bar: 10 µm.

are differentiated from these *Isospora* spp. by the less elongated shape of the sporocysts, smaller and more delicate sub-Stieda body and, mainly, by the presence of the micropyle and rough oöcyst wall which were added in the current redescription.

Phylogenetic analysis

DNA amplification of the oöcyst of *I. feroxis* showed a clear band of *c.*250 bp. Phylogenetic analysis included 20 sequences for avian *Isospora* spp. available on GenBank (Fig. 3). *Toxoplasma gondii* (Nicolle & Manceaux, 1908) was used as the outgroup. *Isospora feroxis* sat apart into a large clade containing *Isospora* spp. from thrushes, warblers, honeyeaters, buntings, longspurs, starlings, white-eyes, but also containing *Isospora* spp. from suboscine hosts (Tyranni), which is the Suborder of *T. sulphurescens* and *M. ferox*. *Isospora feroxis* had the highest similarities of 97% with *Isospora massardi* Lopes, Berto, Luz, Galvão, Ferreira & Lopes, 2014 from the yellow-legged thrush *Turdus flavipes* (Vieillot) and *Isospora manorinae* Yang, Brice, Jian & Ryan, 2016 from the yellow-throated miner *Manorina flavigula* (Gould).

Discussion

The description of coccidian species is fundamentally based on the morphology of the oöcysts; although ecological, biogeographical, pathological and molecular complementations are important for the characterisation of a species (Duszynski & Wilber, 1997; Tenter et al., 2002; Berto et al., 2014). In this context, Duszynski & Wilber (1997) established that the identifications must be based on the comparative morphology between the coccidian species recorded in the same family as the host. Since then, the most complete and reliable studies on coccidian taxonomy from wild birds have demonstrated specificity at the host family level (Berto et al., 2013); however, sometimes the number of coccidian species recorded in a host-family is very low or non-existent (e.g., Cardinalidae), making it necessary to compare with coccidian species described from higher taxonomic levels of the host, such as Superfamily, Parvorder or Infraorder. In addition, the divergences in the systems of classification of Aves make it difficult to identify coccidians based on host-family specificity. The classifications of Del Hoyo & Collar (2016) and Piacentini et al. (2015) that supported the present study classify the tyrant-flycatchers as a Family and Superfamily, respectively; therefore, despite this divergence in level and classifications in different families by Piacentini et al. (2015), there were no

Table 1 Comparative morphology of *Isospora* spp. recorded from New World tyrant-flycatchers (Tyrannoidea)

Species	Host	Reference	Oöcyst					Sporocyst					
			Shape	Size (µm)	Shape index	Polar granule	Wall	Micropyle	Shape	Size (µm)	Shape index	Stieda body	Sub-Stieda body	Sporocyst residuum
***Isospora feroxis* Berto, Luz, Flausino, Ferreira & Lopes, 2009**	*Mytiarchus ferox* (Gmelin) (Tyrannidae)	Berto et al. (2009a)	subspheroidal	18–20 × 17–20 (18.7 × 18.0)	1.0–1.1 (1.1)	usually 2	**low roughness**	**present**	ovoidal	11–13 × 8–10 (11.7 × 8.5)	1.0–1.5 (1.4)	flattened, (0.3 × 1.2)	prominent, (1.2 × 2.5)	diffuse
	M. ferox	**present study**	**subspheroidal**	**21–23 × 20–23 (21.9 × 21.4)**	**1.0–1.1 (1.03)**	**1-3 (usually 2 bonded)**	**low to moderate roughness**	**present**	**ovoidal to ellipsoidal**	**14–15 × 9–10 (14.8 × 10.1)**	**1.4–1.6 (1.47)**	**flattened to half-moon-shaped, 0.5–0.7 × 1.3–2.2 (0.6 × 1.7)**	**rounded to trapezoidal, 1.0–1.8 × 2.4–3.3 (1.3 × 2.9)**	**diffuse**
	***Tolmomyias sulphurescens* Spix (Rhynchocyclidae)**			**18–23 × 18–22 (20.5 × 19.8)**	**1.0–1.1 (1.04)**					**11–15 × 8–10 (13.1 × 9.1)**	**1.3–1.6 (1.45)**			
Isospora mionectesi Berto, Flausino, Luz, Ferreira & Lopes, 2009	*Mionectes rufiventris* Cabanis (Rhynchocyclidae)	Berto et al. (2009b)	ellipsoidal	23–31 × 19–23 (28.3 × 21.2)	1.2–1.4 (1.3)	1-2	smooth	absent	elongate-ellipsoidal	17–22 × 10–13 (19.7 × 11.7)	1.6–1.8 (1.7)	rounded, (0.8 × 1.1)	prominent, (1.4 × 2.1)	compact, subspherical
Isospora attilae Rodrigues, Silva, Lopes, Berto, Luz, Ferreira & Lopes, 2015	*Attila rufus* (Vieillot) (Tyrannidae)	Rodrigues et al. (2015)	subspheroidal to elipsoidal	18–22 × 18–21 (20.3 × 19.0)	1.0–1.2 (1.07)	1-2	smooth	absent	ellipsoidal	12–15 × 7–9 (13.5 × 7.9)	1.6–1.9 (1.7)	knob like, (1.0 × 2.0)	rounded to trapezoidal, (2.5 × 4.0)	diffuse
Isospora lopesi Silva-Carvalho & Berto, 2018	*Platyrinchus mystaceus* Vieillot (Platyrinchidae)	Silva-Carvalho et al. (2018)	subspheroidal to ovoidal	18–24 × 18–22 (20.6 × 19.7)	1.0–1.2 (1.05)	1	smooth	absent	ellipsoidal	12–16 × 8–11 (14.4 × 8.6)	1.5–1.9 (1.7)	flattened to half-moon-shaped, (1.0 × 2.5)	rounded, (2.0 × 2.5)	diffuse

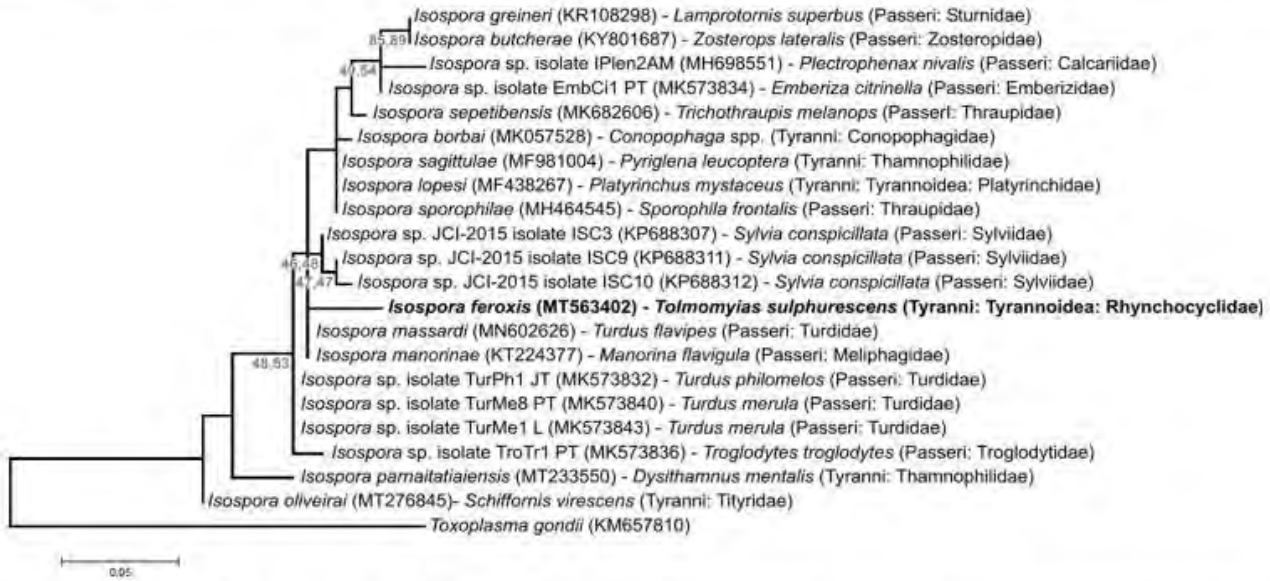

Fig. 3 Maximum likelihood tree estimated from the COI sequences. Numbers at nodes represent bootstrap support (1,000 replicates; only values > 40% shown) for Neighbor-Joining and Maximum Likelihood, respectively. The scale-bar represents the number of nucleotide substitutions per site.

differences in the organization and comparison of coccidians from tyrant-flycatchers that are traditionally related (Berto et al., 2011).

The oöcysts of the original description of *I. feroxis* and those of *M. ferox* and *T. sulphurescens* of the present study differed in measures. These morphometric differences were mainly observed from the low number of oöcysts measured from single hosts *M. ferox*, both in Berto et al. (2009a) and in present study (Table 1). In contrast, the 71 measured oöcysts from 5 *T. sulphurescens* reached a wide range of measures, which is compatible with the largest and smallest oöcysts of *M. ferox*. Thus, it can be concluded that *I. feroxis* has a wide range of measurements detected when observing oöcysts from several hosts. These differences in the size of oöcysts shed from different hosts are natural and already established in the scientific literature as a result of biological and ecological factors (Duszynski, 1971; Fayer, 1981; Berto & Lopes, 2020).

The redescription from the photosyntypes of *I. ferox* proposed in the present study is based on the observation of oöcysts with micropyle (Fig. 2a, b), which were not identified in the original description by Berto et al. (2009a). Probably, the low number of 10 oöcysts observed in Berto et al. (2009a) must have favored the non-observance of the micropyle, since this characteristic feature is observed only in certain positions of the oöcysts (Fig. 2e–g, i, j), especially when they are subspherical (Berto et al., 2014). Thus, this redescription highlights the importance of prioritizing the description of coccidian species from a large number of oöcysts, preventing that certain characteristic features are not observed for the description. In this same sense, the original description by Berto et al. (2009a), did not identify oöcysts with a rough wall. In fact, the oöcysts observed in the current study had a low to moderate roughness, and in one of the hosts *T. sulphurescens*, only oöcysts with very low roughness were predominantly observed, similar to the photosyntypes of *I. ferox*. Therefore, these results reinforce that the description of new coccidian species from a single host specimen should be avoided, ensuring that all possible details of a coccidian species are observed.

Isospora feroxis is the sixth species identified from suboscine birds to have a deposition of COI gene sequence on the GenBank. Even so, few phylogenetic conclusions are observed from the cladogram shown in Fig. 3. *Isospora feroxis* was closer to *Isospora* spp. from thrushes, honeyeaters and warblers than from species of the same parvorder, such as *Isospora oliveirai* Ortúzar-Ferreira & Berto, 2020, and Superfamily/Family, such as *I. lopesi*. At the same time,

Isospora feroxis was as close to coccidians from the Neotropical region, such as *I. massardi*, as well as to coccidian species related to endemic birds in Oceania, such as *I. manorinae*. In fact, this 257 bp fragment of the COI gene has not been totally suitable for the delimitation of coccidian species of passerines, despite that it was pioneered in the work of Dolnik et al. (2009), it has the largest number of deposits at GenBank and, until recently, it has been recommended for phylogenetic studies (Yang et al., 2015). In this sense, the latest works on molecular characterisation of coccidians of Passeriformes has shown that longer sequences and multiple genes are more conclusive in the phylogenetic analyses (Yang et al., 2021). In any case, the molecular analysis confirmed the differences already observed in the morphology of the oöcysts of *I. feroxis* and *I. lopesi*, since these species were genotypically different in 9 base pairs (4.4%) by the COI sequences.

Finally, based on the morphological and molecular features described above, *I. feroxis* is redescribed in the present study, documenting a new host, *T. sulphurescens*, and a new locality, the Itatiaia National Park, in addition to the type-host *M. ferox* in the Marambaia Island, southeastern Brazil.

Acknowledgements We are thankful to staff at the Parque Nacional do Itatiaia, mainly to the research coordinator Dr Léo Nascimento; and the Brazilian Navy, especially to the command of CADIM (Centro de Adestramento da Ilha da Marambaia); that allowed us to access and use some facilities during the expeditions.

Funding This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ). CNO-F has a scholarship from CNPq (Grant/Award Number: 120107/2018-0). JOM has a scholarship from CNPq/UFRRJ (Grant/Award Number: PIB875-2019). MSO has a scholarship from CAPES (Grant/Award Number: 001). BPB has a fellowship from CNPq (Grant/Award Number: 303899/2019-0) and from FAPERJ (Grant/Award Number: E-26/202.797/2019).

Data availability All data generated or analyzed during this study are included in the article.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Field-collecting permits were issued by SISBIO/ICMBio (licenses 45200-1; 49605-1; 54951-1) and CEUA/UFRRJ (protocols IV-036/2014; ICBS-008/2015; IV-6606250616). All applicable institutional, national and international guidelines for the care and use of animals were followed.

References

Berto, B. P., Luz, H. R., Flausino, W., Ferreira, I., & Lopes, C. W. G. (2009a). New species of *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) from the short-crested flycatcher *Myiarchus ferox* (Gmelin) (Passeriformes: Tyrannidae) in South America. *Systematic Parasitology, 74,* 75–80.

Berto, B. P., Flausino, W., Luz, H. R., Ferreira, I., & Lopes, C. W. G. (2009b). *Isospora mionectesi* sp. nov. (Apicomplexa, Eimeriidae) from the grey-hooded flycatcher. *Mionectes rufiventris in Brazil. Acta Parasitologica, 54,* 301–304.

Berto, B. P., Flausino, W., McIntosh, D., Teixeira-Filho, W. L., & Lopes, C. W. G. (2011). Coccidia of New World passerine birds (Aves: Passeriformes): a review of *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae). *Systematic Parasitology, 80,* 159–204.

Berto, B. P., & Lopes, C. W. G. (2013). Distribution and dispersion of coccidia in wild passerines of the Americas. In L. Ruiz & L. Iglesias (Eds.), *Birds: evolution and behavior, breeding strategies, migration and spread of disease* (pp. 47–66). New York: Nova Science Publishers.

Berto, B. P., McIntosh, D., & Lopes, C. W. G. (2014). Studies on coccidian oocysts (Apicomplexa: Eucoccidiorida). *Revista Brasileira de Parasitologia Veterinária, 23,* 1–15.

Berto, B. P., & Lopes, C. W. G. (2020). Coccidia of wild birds as ecological biomarkers: Some approaches on parasite-host-environment interaction. *Journal of Parasitology, 106,* 707–713.

Del Hoyo, J., & Collar, N. J. (2016). *HBW and BirdLife International Illustrated Checklist of the Birds of the World, Volume 2: Passerines.* Cambridge: Lynx Edicions, Barcelona & BirdLife International, 1013 pp.

Dolnik, O. V., Palinauskas, V., & Bensch, S. (2009). Individual oocysts of *Isospora* (Apicomplexa: Coccidia) parasites from avian feces: from photo to sequence. *Journal of Parasitology, 95,* 169–174.

Duszynski, D. W. (1971). Increase in size of *Eimeria separata* oocysts during patency. *Journal of Parasitology, 57,* 948–952.

Duszynski, D. W., & Wilber, P. G. (1997). A guideline for the preparation of species descriptions in the Eimeriidae. *Journal of Parasitology, 83,* 333–336.

Fayer, R. (1980). Epidemiology of protozooan infection: the Coccidia. *Veterinary Parasitology, 6,* 75–103.

ICMBIO. (2016). Parque Nacional do Itatiaia. http://www.icmbio.gov.br/parnaitatiaia. Accessed 13 may 2020.

Piacentini, V. Q., Aleixo, A., Agne, C. E., Maurício, G. N., Pacheco, J. F., Bravo, G. A., et al. (2015). Annotated checklist of the birds of Brazil by the Brazilian Ornithological Records Committee / Lista comentada das aves do

Brasil pelo Comitê Brasileiro de Registros Ornitológicos. *Revista Brasileira de Ornitologia, 23*, 90–298.

Rodrigues, M. B., Silva, L. M., Lopes, B. do B., Berto, B. P., Luz, H. R., Ferreira, I., & Lopes, C. W. G. (2015). A new species of *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) from the grey-hooded attila *Attila rufus* Vieillot, 1819 (Passeriformes: Tyrannidae) on the Marambaia Island, Brazil. *Zootaxa, 4034*, 193–196.

Silva-Carvalho, L. M., Pastura, D. G. N., Gomes, J. V., Siqueira, P. B., Rodrigues, M. B., Lima, V. M., et al. (2018). *Isospora lopesi* n.sp. (Apicomplexa: Eimeriidae) from the eastern white-throated spadebill *Platyrinchus mystaceus* Vieillot (Passeriformes: Tyranni: Tyrannidae) in South America. *Systematic Parasitology, 95*, 455–463.

Stotz, D. F., Fitzpatrick, J. W., Parker, T. A., III, & Moskovits, D. K. (1996). *Neotropical Birds: Ecology and Conservation* (p. 478). Chicago: Universidade de Chicago Press.

Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution, 24*, 1596–1599.

Tenter, A. M., Barta, J. R., Beveridge, I., Duszynski, D. W., Mehlhorn, H., Morrison, D. A., et al. (2002). The conceptual basis for a new classification of the coccidian. *International Journal for Parasitology, 32*, 595–616.

Tundisi, J. G., & Matsumura-Tundisi, T. (2008). Biodiversity in the Neotropics: ecological, economic and social values. *Brazilian Journal of Biology, 68*, 913–915.

Williams, R. B., Thebo, P., Marshall, R. N., & Marshall, J. A. (2010). Coccidian oöcysts as type-specimens: long-term storage in aqueous potassium dichromate solution preserves DNA. *Systematic Parasitology, 76*, 69–76.

Yang, R., Brice, B., Elliot, A., & Ryan, U. (2015). *Isospora serinuse* n. sp. (Apicomplexa: Eimeriidae) from a domestic canary (*Serinus canaria forma domestica*) (Passeriformes: Fringillidae) in Western Australia. *Experimental Parasitology, 159*, 59–66.

Yang, R., Brice, B., Liu, Q., Berto, B. P., Austen, J., & Ryan, U. (2021). Morphological and genetic characterization of the first *Isospora* species (*I. lugensae* n. sp.) from a Kerguelen petrel (*Lugensa brevirostris*). *Parasitology Research, 120*, 1037–1047.

Parasitology Research (2021) 120:2585–2593
https://doi.org/10.1007/s00436-021-07140-9

PROTOZOOLOGY - ORIGINAL PAPER

Distribution, redescription, and molecular identification of *Isospora striata* McQuistion et al. 1997 (Eimeriidae), from woodcreepers (Dendrocolaptidae) in South America

Mariana S. Oliveira[1] · Ericson R. de Mello[2] · Sergian V. Cardozo[3] · Águida A. Oliveira[4] · Viviane M. Lima[4] · Ildemar Ferreira[4] · Bruno P. Berto[4]

Received: 12 February 2021 / Accepted: 24 March 2021 / Published online: 2 April 2021

Abstract
Woodcreepers are passerines of the family Dendrocolaptidae, which have a high forest dependency. The current work aimed to redescribe *Isospora striata* McQuistion et al. 1997, from two new hosts in protected areas in Brazil, revealing new localities of parasitism, in addition to providing preliminary genotypic identifications via sequencing of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene from both host species. *Isospora striata* has oocysts that are subspheroidal to ovoidal, 19.4 × 16.8 μm with smooth wall. Oocyst residuum is absent, but micropyle and polar granules are present. Sporocysts are ovoidal, 13.6 × 8.3 μm, with both Stieda and sub-Stieda bodies. Sporocyst residuum is present and sporozoites with refractile body, nucleus, and striations. The morphological study and the 100% similarity in sequencing of the COI gene between samples of different dendrocolaptid species confirmed the identification of a single species, supporting the identification of *I. striata* in the Brazilian Atlantic forest and consequently the wide distribution of this coccidian species in the Neotropical Region.

Keywords Taxonomy · Morphology · Sequencing · Coccidia · Oocysts · Passeriformes · Parque Nacional de Itatiaia · Parque Nacional da Serra dos Órgãos · Brazil

Introduction

Woodcreepers are passerines of the family Dendrocolaptidae, which brings together 52 species distributed predominantly in forest environments in the Neotropical Region (Marantz et al. 2003). Dependence on forest environments causes dendrocolaptid species to suffer population decline and even local extinction in altered forests and forest fragments (Marantz et al. 2003; IUCN 2020). In the Atlantic Forest of the Southeast and South of Brazil, several studies confirm this vulnerability of the woodcreepers to anthropogenic changes, notably forest fragmentation (Aleixo and Vielliard 1995; Christiansen and Pitter 1997; Bornschein and Reinert 2000).

These birds feed predominantly on large insects, small vertebrates, snails, and bird eggs that nest in tree cavities. They also regularly follow mixed flocks and army ants, foraging in all strata as dominant species (Sick 1997; Piacentini et al. 2015). This predominantly insectivorous feeding habit potentially reduces these birds the fecal-oral transmission of parasites, unlike frugivorous birds (Dolnik et al. 2010). Among the various parasites in this context, the coccidian protozoans can be highlighted due to its great importance for biodiversity and conservation of birds.

To date, six coccidian species are recorded from Neotropical woodcreepers, but none of them in Brazil. In this sense, the current work aimed to redescribe *Isospora striata* McQuistion et al. 1997, from two new hosts in protected areas in Brazil,

Section Editor: Nawal Hijjawi

✉ Bruno P. Berto
berto.ufrrj@gmail.com

[1] Programa de Pós-Graduação em Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7, Seropédica, RJ 23897-000, Brazil

[2] Programa de Pós-Graduação em Ciências Veterinárias, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7, Seropédica, RJ 23897-000, Brazil

[3] Programa de Pós-Graduação em Biomedicina Translacional, Universidade do Grande Rio, Rua Professor José de Souza Herdy 1160, Duque de Caxias, RJ 25071-202, Brazil

[4] Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7, Seropédica, RJ 23897-000, Brazil

revealing new localities and the wide distribution of this coccidian species in the Neotropical Region. Additionally, the present study will provide preliminary genotypic identifications via sequencing of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene from both host species.

Materials and methods

Sample collection

Eight expeditions were conducted in two Brazilian federal conservation units in Southeastern Brazil: (1) Itatiaia National Park, a protected area with a high degree of vulnerability, located in the Serra da Mantiqueira on the border of the States of Rio de Janeiro, Minas Gerais, and São Paulo (ICMBIO 2020a); and (2) Serra dos Órgãos National Park, another protected area created for biodiversity conservation of the Serra do Mar in the mountainous region of the State of Rio de Janeiro (ICMBIO 2020b). The first six expeditions were conducted on March (22°27′40.3″S, 44°35′31.9″W) and April (22°27′52.0″S, 44°36′26.0″W) 2015, October (22°27′40.3″S, 44°35′31.9″W) 2016, April (22°27′20.6″S, 44°36′28.6″W) 2017, and May (22°27′40.3″S, 44°35′31.9″W) and August (22°26′57.0″S, 44°36′25.0″W) 2018 in the Itatiaia National Park, and the last two expeditions were conducted on February (22°27′23.8″S, 42°59′58.7″W) and August (22°27′29.0″S, 43°00′08.8″W) 2019 in the Serra dos Órgãos National Park. Mist nets were used for the capture of the birds. Sixteen plain-winged woodcreepers *Dendrocincla turdina* (Lichtenstein, 1820) in the Itatiaia National Park and two white-throated woodcreepers *Xiphocolaptes albicollis* (Vieillot, 1818) in the Serra dos Órgãos National Park were captured. The captured birds were specifically identified (Sigrist 2014) and photographed. Subsequently, the birds were kept in individual boxes lined with clean paper until defecation, when they were released at the same place of capture. Each fresh droplet of feces from each individual bird was placed individually in a centrifuge tube with a potassium dichromate 2.5% ($K_2Cr_2O_7$) solution.

Morphological analyses

Samples were transported to the Laboratório de Biologia de Coccidios, Universidade Federal Rural do Rio de Janeiro (UFRRJ). Samples were incubated in the centrifuge tubes and regularly oxygenated by shaking, at room temperature (~25 °C) for 10 days or until ~70% of the oocysts were sporulated. Oocysts were isolated by flotation in Sheather's sugar solution (Specific gravity: 1.20) and examined microscopically using the technique described by Duszynski and Wilber (1997) and Berto et al. (2014a). Morphological observations, line drawings, photomicrographs, and measurements were made using an Olympus BX binocular microscope (Olympus Optical, Tokyo, Japan) coupled to a digital camera Eurekam 5.0 (BEL Photonics, Monza, Italy). Line drawings were edited using two software applications from CorelDRAW® (Corel Draw Graphics Suite, Version 11.0, Corel Corporation, Canada), i.e., Corel DRAW and Corel PHOTO-PAINT. All measurements are in micrometers and are given as the range followed by the mean in parentheses.

Molecular analyses

Individual oocysts identified with the same characteristic features under light microscopy were isolated, resuspended in PBS, and washed by centrifuging until the supernatant became clear (Dolnik et al. 2009). DNA was extracted from the oocysts using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, São Paulo, Brazil) according to the manufacturer's instructions. In order to fully lyse the oocysts, four freeze-thaw cycles were applied prior to the DNA extraction. The PCR amplification for the COI gene was carried out using a nested PCR, as previously described by Dolnik et al. (2009) and Yang et al. (2015). The external primers COIbF1 (5′-GWT CAT TAG TAT GGG CAC ATC A-3′) and COIbR1 (5′-CCA AGA GAT AAT ACR AAR TGG AA-3′) produced a PCR product of 302 bp in size. The internal primes COIbF2 (5′-GGG CAC ATC ATA TGA TGA C-3′) and COIbR2 (5′-ATA GTA TGT ATC ATG TAR WGC AA-3′) produced an amplicon of 257 bp in size. The PCR reaction contained 12.5 μL of GoTaq® G2 Hot Start Colorless Master Mix (Promega) (1×), 0.25 μL of each primer (0.2 μM), 3 μL of DNA (for primary reaction) or 3 μL of primary PCR product (for the secondary reaction), and 9 μL of nuclease-free water. Both primary and secondary PCR were conducted using the same cycling conditions: 1 cycle of 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 47 °C for 45 s, and 72 °C for 1 min and a final extension of 72 °C for 5 min. The amplicons from the second round of PCR were purified using the Qiagen MinElute PCR Purfication (Qiagen, São Paulo, Brazil).

DNA sequence analyses

All PCR products were sequenced using the PCR forward and reverse primers by Ludwig Biotechnology, using an ABI-Prism 3500 Genetic Analyzer (Applied Biosystems, Foster City, California) for Sanger sequencing. The results of the sequencing reactions were analyzed and edited using the program Chromas 2.6. Sequences were compared to each other and with other coccidian parasites available on the GenBank database using the Basic Local Alignment Search Tool (BLAST). Phylogenetic trees were constructed for coccidian species at the COI sequences for additional isolates from GenBank. Alignment and parsimony analyses were conducted using MEGA version 7 (Tamura et al. 2007). The evolutionary history was inferred using the neighbor joining (NJ)

and maximum likelihood (ML) methods, and the distances were computed using the Tamura-Nei method based on model selection using ModelTest in MEGA 7. Initial trees for the heuristic search were obtained automatically by applying neighbor joining and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach and then selecting the topology with superior log likelihood value. Bootstrap analyses were conducted using 1000 replicates to assess the reliability of inferred tree topologies.

Results

Prevalence and identification

Sixteen *D. turdina* from the Itatiaia National Park were examined, and 9 were positive for coccidian oocysts. The two *X. albicollis* captured in the Serra dos Órgãos National Park were also positive. These oocysts from both host species, after sporulation, were morphologically identified as *I. striata*. This material is described below.

- *Isospora striata* McQuistion et al. 1997 (Figs. 1 and 2a–j)
- Kingdom: Chromista Cavalier-Smith, 1981
- Phylum: Miozoa Cavalier-Smith, 1987
- Infraphylum: Apicomplexa Levine, 1970
- Class: Coccidiomorphea Doflein, 1901
- Subclass: Coccidia Leuckart, 1879
- Family: Eimeriidae Minchin, 1903
- Genus: *Isospora* Schneider, 1881

Oocyst (n = 88) subspheroidal to ovoidal, 16–23 × 13–21 (19.4 × 16.8); length/width (L/W) ratio 1.0–1.4 (1.16). Wall bi-layered, 1.1–1.5 (1.3) thick, outer layer smooth, *c.*2/3 of total thickness. Micropyle delicate or inconspicuous, 2.8–7.1 (4.2) wide. Oocyst residuum is absent, but 1–3 polar granules are present. Sporocyst ovoidal, 11–16 × 6–10 (13.6 × 8.3); L/W ratio 1.5–2.0 (1.64). Stieda body is present, protruding, rounded to knob-like, 1.1–2.1 high × 1.2–1.6 wide (1.3 × 1.4). Sub-Stieda body is present, rectangular to rounded, and lying directly beneath the Stieda body, 1.0–1.4 high × 1.4–2.2 wide (1.2 × 1.9). Para-Stieda body is absent. Sporocyst residuum is present, consisting of granules partially bound and/or diffused. Sporozoites vermiform, with posterior refractile body, central nucleus, and striations.

Taxonomic summary

Hosts: *Dendrocincla turdina* (Lichtenstein, 1820) (Aves: Passeriformes: Tyranni: Dendrocolaptidae: Sittasominae), plain-winged woodcreeper; *Xiphocolaptes albicollis* (Vieillot, 1818) (Aves: Passeriformes: Tyranni: Dendrocolaptidae: Dendrocolaptinae), white-throated woodcreeper.

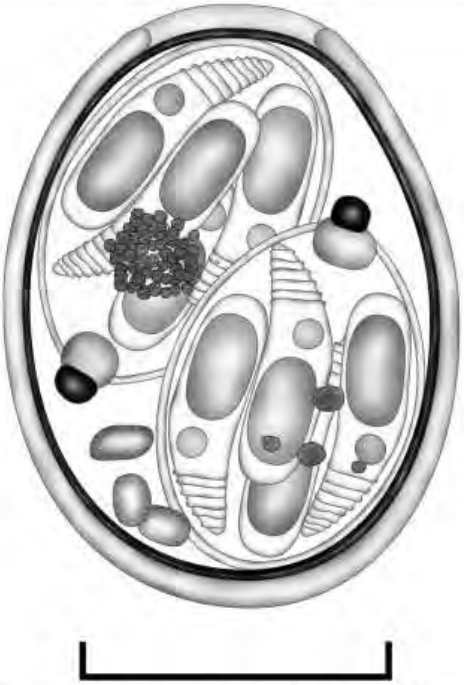

Fig. 1 Composite line drawing of the sporulated oocyst for redescription of *Isospora striata* from woodcreepers in the Brazilian Atlantic forest. Scale bar: 10 μm

Localities: Itatiaia National Park (22°27′S, 44°35′W) and Serra dos Órgãos National Park (22°27′S, 43°00′W), both from Southeastern Brazil.

Specimens: Photomicrographs, line drawing, and oocysts in 2.5% $K_2Cr_2O_7$ solution (Williams et al. 2010) are deposited at the Museu de Zoologia at the Universidade Federal Rural do Rio de Janeiro, Brazil, under accession numbers MZURPTZ2020026 (*D. turdina*) and MZURPTZ2020027 (*X. albicollis*). Photomicrographs are also deposited and available (http://r1.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under repository numbers 110/2020 (*D. turdina*) and 111/2020 (*X. albicollis*). Photovouchers of the host specimens are deposited in the same collection.

Representative DNA sequence: DNA amplification of the COI gene showed clear bands around ~250 bp. Representative sequences were deposited in the GenBank database under the accession numbers MW582619 (*D. turdina*) and MW582620 (*X. albicollis*).

Site of infection: Unknown.

Prevalence: 61% (11/18) in total; 56% (9/16) for *D. turdina*; and 100% (2/2) for *X. albicollis*.

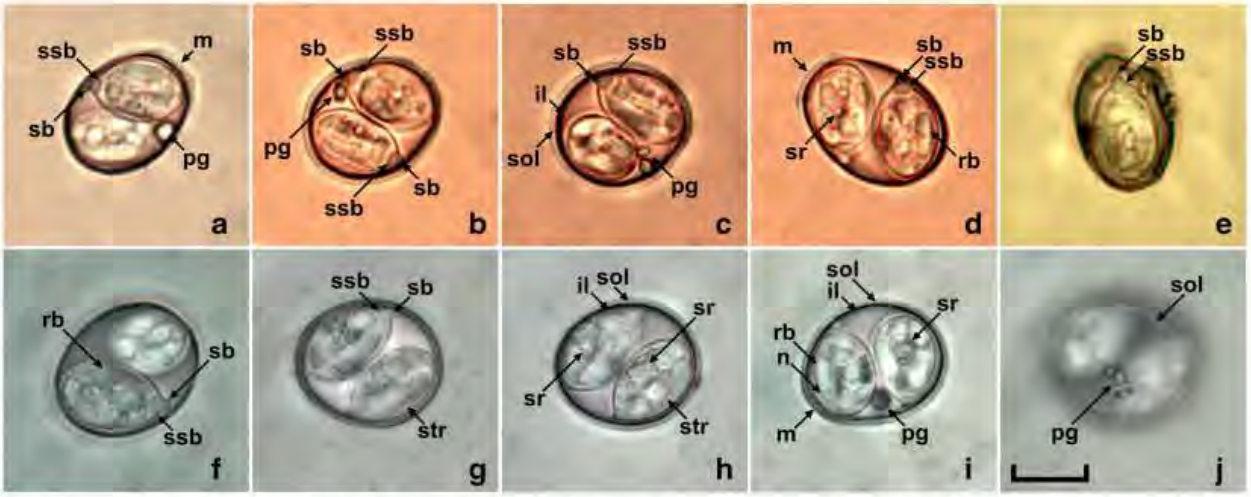

Fig. 2 Photomicrographs of *Isospora striata* recovered from plain-winged woodcreepers *Dendrocincla turdina* (a–e) and white-throated woodcreepers *Xiphocolaptes albicollis* (f–j). Note the inner (il) and the smooth outer (sol) layer of the oocyst wall, micropyle (m), nucleous (n), polar granules (pg), refractile body (rb), sporocyst residuum (sr), Stieda body (sb), and sub-stieda (ssb) bodies and striations (str). Scale bar: 10 μm

Phylogenetic analysis

Phylogenetic analysis based on the COI gene included sequences from coccidians available in GenBank (Fig. 3). *Eimeria tenella* (Railliet and Lucet, 1891) was used as the outgroup. *Isospora striata* from *D. turdina* and *X. albicollis* were 100% identical and sat in a clade with the similarity of 99% with *Isospora feroxis* Berto, Luz, Flausino, Ferreira, and Lopes, 2009, which is a parasite of tyrant-flycatchers (Tyranni: Tyrannoidea) in Brazil (Ortúzar-Ferreira et al. 2021). *Isospora striata* was also close to other *Isospora* spp. of neotropical suboscine passerines (Tyranni), such as *Isospora saggitulae* McQuistion and Capparella, 1992, of antbirds (Thamnophilidae) with 96% similarity (Silva-Carvalho et al. 2018) but also with neotropical oscine passerines (Passeri), such as *Isospora massardi* Lopes, Berto, Luz, Galvão, Ferreira and Lopes, 2014, of thrushes (Turdidae) with 97% similarity (Genovez-Oliveira et al. 2020), in addition to

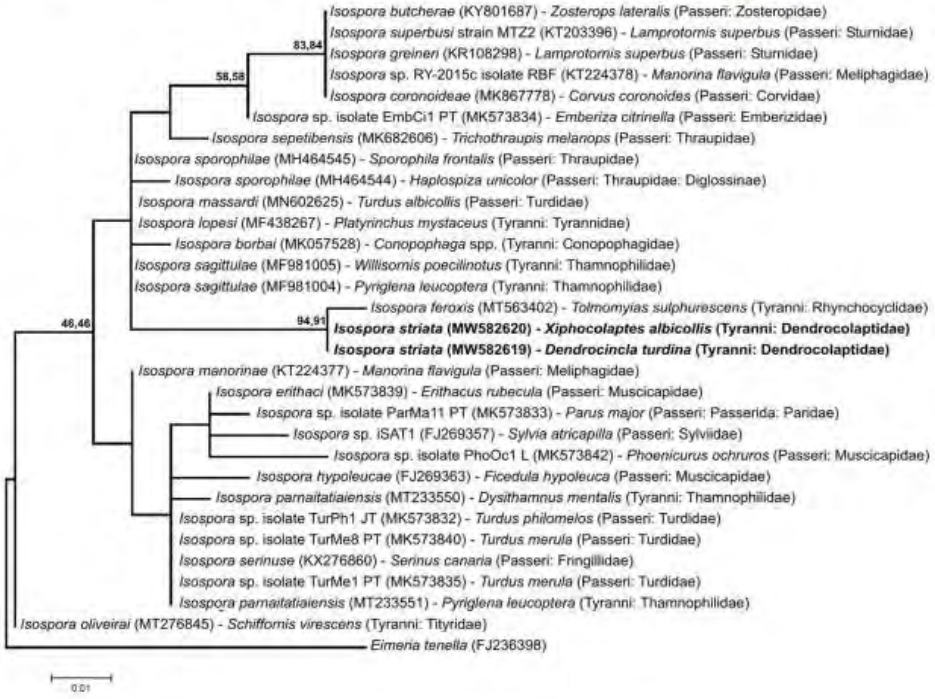

Fig. 3 Maximum likelihood tree estimated from the COI gene sequences of *Isospora* species. Numbers at nodes represent bootstrap support 1000 replicates (> 50%) for neighbor joining (NJ) and maximum likelihood (ML), respectively. Scale bar represents the number of nucleotide substitutions per site

Table 1 Comparative morphology of *Isospora* spp. recorded from woodcreepers (Dendrocolaptidae)

Coccidia	Hosts	Locality	References	Oocysts						
				Shape	Length (μm)	Width (μm)	L/W ratio	Wall	Residuum	Micropyle
Isospora concentrica McQuistion and Caparella, 1995	*Dendrocolaptes certhia* (Boddaert, 1783)	South America, Equador	McQuistion and Caparella (1995)	Ovoid	24–30 (26.9)	21–25 (22.7)	1.04–1.38	Smooth	Absent	Absent
Isospora magna McQuistion and Caparella, 1995	*D. certhia*	South America, Equador	McQuistion and Caparella (1995)	Ovoidal	26.0–31.0 (29.7)	23.0 – 26.0 (24.9)	1.08–1.30 (1.20)	Smooth	Absent	Absent
Isospora ocellati McQuiston, Walden and Caparella, 1997	*Xiphorhynchus ocellatus* (Spix, 1824)	South America, Equador	McQuistion et al. (1997)	Ovoidal	18–21 (20.0)	15–19 (17)	1–1.3 (1.2)	Smooth	Absent	Absent
Isospora striata McQuiston, Walden and Caparella, 1997	*X. ocellatus* (Spix, 1824)	South America, Equador	McQuistion et al. (1997)	Ovoidal	l6–20 (18)	15–17 (16)	1.1–1.2 (1.2)	Smooth	Absent	Absent
	Xiphocolaptes albicollis (Vieillot, 1818); *Dendrocincla turdina* (Lichtenstein, 1820)	South America, Brazil	Current work	Subspheroidal to ovoidal	16–23 (19.4)	13–21 (16.8)	1.0–1.4 (1.2)	Smooth	Absent	Present, inconspicuous
Isospora ubique McQuistion and Caparella, 1997	*Glyphorynchus spirurus* (Vieillot, 1819)	South America, Equador	McQuistion and Caparella (1997)	Subspherical	21 –27 (23.4)	19–24 (21.8)	1.00–1.23 (1.07)	Smooth	Absent	Absent
Isospora dendrocinclae McQuistion, Galewsky and Caparella, 2010	*Dendrocincla merula* (Lichtenstein, 1820)	South America, Guyana	McQuiston et al. (2010)	Subspherical to ovoid	15–23	14.5–19	1.1–1.3 (1.2)	Smooth	Absent	Absent

Coccidia	Oocysts	Sporocysts							Sporozoites		
	Polar granule	Shape	Length (μm)	Width (μm)	L/W ratio	Stieda body	Substieda body	Residuum	Refractile body	Nucleus	Striations
Isospora concentrica McQuistion and Caparella, 1995	Present	Ovoid to ellipsoidal	15.0–18.0 (17.2)	9.0–12.0 (11.0)	1.42–1.70 (1.56)	Block-shaped	Ovoidal or bubble–shaped	Present, compact	Present, 1 posterior	Present	Present
Isospora magna McQuistion and Caparella, 1995	Subspherical to ovoid	Ovoidal	15.0–20.0 (15.8)	11.0–14.0 (12.6)	1.07–1.82 (1.28)	Broad, dome-like	Inconspicuous	Composed of uniform	Present, 2	Present	Absent
Isospora ocellati McQuiston, Walden and Caparella, 1997	Ovoid to subspherical	Ovoidal	11–13 (12)	7–9 (8)	1.2–1.7 (1.5)	Dome or bubble-like	Elipsoidal	Composed	Present, 1 posterior	Present	Absent
Isospora striata McQuiston,	Spherical	Ovoidal	1l–12 (11)	7–8 (8)	1.4–1.5 (1.4)	Protruding and nipple-like	Rectangular	Compact, spherical	Present, 1 posterior	Present	Present
		Ovoidal	11–16 (13.6)	6–10 (8.3)	1.5–2.0 (1.6)					Present	Present

Table 1 (continued)

Walden and Caparella, 1997	Subspherical to ellipsoidal, 1–3					Protruding, rounded to knob-like	Rectangular to rounded	Compact or diffused	Present, 1 posterior		
Isospora ubique McQuistion and Caparella, 1997	Ovoid	Ovoidal	14–16 (14.8)	9–11 (10.1)	1.36–1.67 (1.46)	Nipple-like	Absent	Composed	Present, 1 posterior	Present	Absent
Isospora dendrocinclae McQuistion, Galewsky and Caparella, 2010	Subspherical	Ovoid	12–14	7–10	1.6–1.8 (1.7)	Small and knob-like	Slightly larger and subglobular	Subspherical	Present, 1 posterior	Present	Absent

other *Isospora* spp. from other distant zoogeographic regions, such as *Isospora coronoideae* Liu et al. 2019, from Australian ravens *Corvus coronoides* Vigors and Horsfield, 1827 (Passeri: Corvidae) from Australia with 95% similarity (Liu et al. 2019).

Discussion

In Brazil, Passeriformes brings together 37 families distributed in all Brazilian biomes (Atlantic Forest, Amazon, Pantanal, Caatinga, Cerrado, and Pampa), comprising more than half of all birds. Dentrocolaptidae comprises 14 genera and 43 species known as "arapaçus" in Brazil (Piacentini et al. 2015). Despite this great diversity, the current work reports for the first time an *Isospora* sp. from Brazilian woodcreepers. The low density and prevalence expected from insectivorous birds (Dolnik et al. 2010) could justify this first report in Brazilian woodcreepers only in 2020; however, contrary to what was expected, the prevalence was reasonably high in the current study (61%), even with the low number of woodcreepers analyzed.

Isospora striata was originally described from ocellated woodcreepers *Xiphorhynchus ocellatus* (Spix, 1824) in the province of Morona-Santiago, about 5 km southwest of Taisha, in Ecuador (McQuiston et al. 1997). The oocysts identified in the current study were morphologically compatible with this original description by McQuistion et al. (1997); with the exception of the presence of the micropyle and other minor differences in size and shape of some characteristic features (Table 1). The micropyle identified in the oocysts of the current work is delicate and inconspicuous, being difficult to observe in most oocysts; therefore, it must have been unobserved by McQuistion et al. (1997). The differences in the morphometry might be justified by the greater number of oocysts measured in the current study, in addition to the greater number of host species and specimens (Sampaio 2002; Berto and Lopes 2020). The morphometric comparison between the oocysts of the two hosts of the current work was suppressed, due to the low number of oocysts and hosts *X. albicollis* in relation to *D. turdina*, which would weaken any statistical analysis. In any case, the morphometric differences between current and original work of McQuistion et al. (1997) were mainly associated with the measured ranges, while the means were closer. In fact, the main characteristic features that guided the identification as *I. striata* in the current work were the striations in the sporozoites and the rectangular sub-Stieda body, which were exceptionally highlighted by McQuistion et al. (1997) for being the main distinctive features of *I. striata* in comparison to the other *Isospora* spp. recorded from Dencrocolaptidae (Table 1).

The hosts of the current study, *D. turdina* and *X. albicollis*, and the host of the original description of *I. striata*,

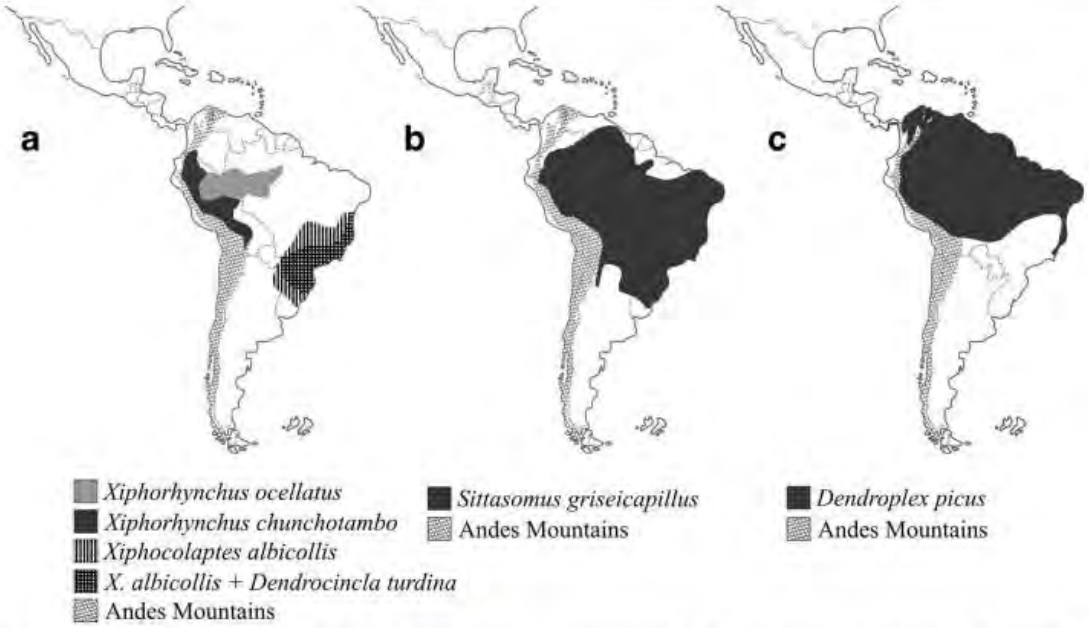

Fig. 4 Geographic range of the dendrocolaptid hosts of *Isospora striata* in the Neotropical Region [based on data from IUCN 2020]. *Xiphorhynchus chunchotambo* and *Xiphorhynchus ocellatus* are distributed in the Amazon. The news hosts *Xiphocolaptes albicollis* and *Dendrocincla turdina* have geographic ranges in the Altlantic Forest in southeastern Brazil (a). *Sittasomus griseicapillus* (b) and *Dendroplex picus* (c) are examples of woodcreepers with wide Neotropical geographic ranges that are sympatric with *X. chunchotambo*, *X. ocellatus*, *X. albicollis*, and *D. turdina*

X. ocellatus, are not sympatric. In fact, the original host identified by McQuistion et al. (1997) is potentially the current Tschudi's woodcreeper *Xiphorhynchus chunchotambo* (von Tschudi, 1844), which was previously classified as a subspecies of *X. ocellatus*. Nevertheless, currently, only *X. chunchotambo* has distribution in Ecuador, while *X. ocellatus* is restricted to the Brazilian and Peruvian Amazon, being slightly sympatric with *X. chunchotambo* in the Brazilian States of Acre and Rondônia (Fig. 4). At the opposite end in South America, *D. turdina* and *X. albicollis* are distributed strictly in the Atlantic Forest.

Thus, the non-sympatry of these hosts could be incompatible with the identification of *I. striata* in the current study, since the transmission of *Isospora* spp. is fecal-oral and, therefore, depends on the minimum sympatry for there to be transmission/dispersion of its oocysts; however, as assumed in the studies by Berto et al. (2014b), Silva et al. (2017), and Silva-Carvalho et al. (2018), susceptible hosts with wide geographic distributions in South America could transmit *I. striata* to a wide range of sympatric susceptible hosts. In this way, the eastern olivaceous woodcreeper *Sittasomus griseicapillus* (Vieillot, 1818) and straight-billed woodcreeper *Dendroplex picus* (Gmelin, 1788) can be potential transmitters/dispersers of *I. striata* in South America, since they have wide geographical distributions and are sympatric with *X. ocellatus*/*X. chunchotambo*, *D. turdina*, and *X. albicollis* (Fig. 4). It is worth mentioning that this assumption is based on the concept of specificity at the host family level, which has been widely accepted in the numerous studies of coccidian taxonomy (Duszynski and Wilber 1997; Berto et al. 2011).

The genotypic similarity of 100% at the COI gene between the samples of *D. turdina* and *X. albicollis* confirms the identification of a single species of these hosts and reinforces the identification of *I. striata* in the Brazilian Atlantic forest, because if this species can parasitize woodcreepers of different species, potentially this species must be widely distributed within the geographic ranges of neotropical woodcreepers (Fig. 4) (Berto and Lopes 2020).

The phylogenetic analysis of Fig. 3 does not allow further conclusions about the origin or aspects of monophyletic groups, since *Isospora* spp. from passerines of different families and suborders, from different and distant zoogeographic regions and with different characteristics sat in the same clades. Indeed, the use of a single gene for genotypic identification of *Isospora* spp. does not allow a more detailed phylogenetic characterization (Yang et al. 2021); in any case, the COI gene has been the most indicated for species confirmation (Ogedengbe et al. 2011, Yang et al. 2015, Silva-Carvalho et al. 2018) and the most used for genotypic and phylogenetic studies of *Isospora* spp., possibly due to the favorable extraction and amplification of mitochondrial genes, which are in a greater number of copies, from individual oocysts (Dolnik et al. 2009).

Finally, based on the morphological and molecular features described above, *I. striata* is redescribed in the current work, documenting two new hosts, *D. turdina* and *X. albicollis*, and new localities in the Brazilian Atlantic forest, the Itatiaia National Park and the Serra dos Órgãos National Park.

Acknowledgements We are thankful to staff at the Parque Nacional do Itatiaia, mainly to the research coordinator Dr Léo Nascimento, and the Parque Nacional da Serra dos Órgãos, mainly to the research coordinator Dr Jorge Nascimento (Julião), who allowed us to access and use some facilities during the expeditions.

Funding This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ). MSO has a scholarship from CAPES (Grant/Award Number: 001). BPB has a fellowship from CNPq (Grant/Award Number: 303899/2019-0) and from FAPERJ (Grant/Award Number: E-26/202.797/2019).

Declarations

Ethical approval Field-collecting permits were issued by SISBIO/ICMBio (licenses 45200; 49605; 54951; 61126) and CEUA/UFRRJ (protocols IV-036/2014; ICBS-008/2015; IV-6606250616). All applicable institutional, national, and international guidelines for the care and use of animals were followed.

Conflict of interest The authors declare no competing interests.

References

Aleixo A, Vielliard J (1995) Composição e dinâmica da avifauna da mata de Santa Genebra, Campinas, São Paulo, Brasil. Rev Bras Zool 12: 493–511

Berto BP, Lopes CWG (2020) Coccidia of wild birds as ecological biomarkers: some approaches on parasite-host-environment interaction. J Parasitol 106:707–713

Berto BP, Flausino W, McIntosh D, Teixeira-Filho WL, CWG L (2011) Coccidia of new world passerine birds (Aves: Passeriformes): a review of *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae). Syst Parasitol 80:159–204

Berto BP, McIntosh D, Lopes CWG (2014a) Studies on coccidian oocysts (Apicomplexa: Eucoccidiorida). Rev Bras Parasitol Vet 23:1–15

Berto BP, Lopes BB, Melinski RD, Souza A, Ribas C, Abreu F, Ferreira I, Lopes CWG (2014b) Coccidial dispersion across *trans-* and *cis-*Andean antbirds (Passeriformes: Thamnophilidae): *Isospora sagittulae* McQuistion and Capparella, 1992 (Apicomplexa: Eimeriidae) from non-sympatric hosts. Can J Zool 92:383–388

Bornschein MR, Reinert BL (2000) Aves de três remanescentes florestais do norte do Estado do Paraná. Rev Bras Zool 17:615–636

Christiansen B, Pitter E (1997) Species loss in a forest bird community near Lagoa Santa in southeastern Brazil. Biol Conserv 80:23–32

Dolnik OV, Palinauskas V, Bensch S (2009) Individual oocysts of *Isospora* (Apicomplexa: Coccidia) parasites from avian feces: from photo to sequence. J Parasitol 95:169–174

Dolnik OV, Dolnik VR, Bairlein F (2010) The effect of host foraging ecology on the prevalence and intensity of coccidian infection in wild passerine birds. Ardea 98:97–103

Duszynski D, Wilber P (1997) A guideline for the preparation of species descriptions in the Eimeridae. J Parasitol 83:333–336

Genovez-Oliveira JL, Oliveira MS, Thode-Filho S, Cardozo SV, Oliveira ÁA, Lima VM, Ferreira I, Berto BP (2020) Morphological and molecular identification of *Isospora massardi* Lopes, Berto, Luz, Galvão, Ferreira & Lopes, 2014 (Chromista: Miozoa: Eimeriidae) from thrushes *Turdus* spp. (Passeriformes: Turdidae) in South America. Parasitol Int 75:102040

ICMBIO (2020a) Parque Nacional do Itatiaia. Available from: <https://www.icmbio.gov.br/parnaitatiaia>. Accessed 02 Jul 2020

ICMBIO (2020b) Parque Nacional da Serra dos Órgãos. Available from: <https://www.icmbio.gov.br/parnaserradosorgaos>. Accessed 02 Jul 2020

IUCN (2020) The IUCN Red List of Threatened Species. Available from: <http://www.iucnredlist.org>. Accessed 02 Aug 2020

Liu D, Brice B, Elliot A, Ryan U, Yang R (2019) *Isospora coronoideae* n. sp.(Apicomplexa: Eimeriidae) from the Australian raven (*Corvus coronoides*)(Passeriformes: Corvidae) (Linnaeus, 1758) in Western Australia. Parasitol Res 118:2399–2408

Marantz CA, Aleixo A, Bevier LR, Patten MA (2003) In: del Hoyo J, Elliott A, Christie D (eds) Family Dendrocolaptidae (Woodcreepers). Handbook of the birds of the world. Lynx Edicions, Barcelona, pp 358–447

McQuistion TE, Capparella A (1995) Two new isosporan parasites (Apicomplexa: Eimeriidae) from the South American woodcreeper *Dendrocolaptes certhia*. Acta Protozool 34:299–302

McQuistion TE, Capparella A (1997) *Isospora ubique*: a new coccidian parasite from the wedge-billed woodcreeper (*Glyphorynchus spirurus*) from South America. Acta Protozool 36:75–78

McQuistion TE, Walden KKO, Capparella AP (1997) Two new species of *Isospora* (Apicomplexa:Eimeriidae) from the ocellated woodcreeper, *Xiphorhynchus ocellatus* (Passeriformes: Dendrocolaptidae). Arch Protistenkd 147:401–404

McQuistion TE, Galewsky S, Capparella AP, Rebling F (2010) *Isospora dendrocinclae* n. sp. (Apicomplexa: Eimeriidae) from the whitechinned woodcreeper (*Dendrocincla merula*) from South America. Acta Protozool 49:121–124

Ogedengbe JD, Hanner RH, Barta JR (2011) DNA barcoding identifies *Eimeria* species and contributes to the phylogenetics of coccidian parasites (Eimeriorina, Apicomplexa, Alveolata). Int J Parasitol 41: 843–850

Ortúzar-Ferreira CN, Mello ER, Melo JO, Oliveira MS, Thode-Filho S, Cardozo SV, Oliveira ÁA, Lima VM, Ferreira I, Berto BP (2021) Redescription and molecular identification of *Isospora feroxis* Berto, Luz, Flausino, Ferreira & Lopes, 2009 (Eimeriidae) from tyrant-flycatchers (Tyrannoidea) in South America. Syst Parasitol [in press]

Piacentini VDQ, Aleixo A, Agne CE, Maurício GN, Pacheco JF, Bravo GA, Brito GRR, Naka LN, Olmos F, Posso S, Silveira LF, Betini GS, Carrano E, Franz I, Lees AC, Lima LM, Pioli D, Schunck F, Amaral FR, Bencke GA, Cohn-Haft M, LFA F, Straube FC, Cesari E (2015) Annotated checklist of the birds of Brazil by the Brazilian Ornithological Records Committee. Rev Brasil Ornitol 23:91–298

Sampaio IBM (2002) Estatística aplicada à experimentação animal. Belo Horizonte, FEP MVZ Editora, 265pp

Sick H (1997) Ornitologia Brasileira. Nova Fronteira, Rio de Janeiro, 862 pp

Sigrist T (2014) Guia de Campo: Avifauna Brasileira. São Paulo, Avis Brasilis, 608 pp

Silva LM, Rodrigues MB, Pinho IF, Lopes BB, Luz HR, Ferreira I, Lopes CWG, Berto BP (2017) Some remarks on the distribution and dispersion of coccidia from icterid birds in South America: *Isospora guaxi* n. sp. and *Isospora bellicosa* Upton, Stamper & Whitaker, 1995 (Apicomplexa: Eimeriidae) from the red-rumped cacique *Cacicus*

haemorrhous (L.)(Passeriformes: Icteridae) in Southeastern Brazil. Syst Parasitol 94:151–157

Silva-Carvalho LM, Pastura DG, Rodrigues MB, Gomes JV, Oliveira MS, Siqueira PB, Genovez-Oliveira JL, Soares SS, Oliveira ÂA, Lima VM, Ferreira I, Berto BP (2018) *Isospora sagittulae* McQuistion & Capparella, 1992 (Apicomplexa: Eimeriidae) from antbirds (Passeriformes: Thamnophilidae) in the Amazon and Atlantic Forest of Brazil: with notes on its distribution and dispersion in the Neotropical region. Parasitol Res 117:2635–2641

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Williams RB, Thebo P, Marshall RN, Marshall JA (2010) Coccidian oöcysts as type–specimens: long–term storage in aqueous potassium dichromate solution preserves DNA. Syst Parasitol 76:69–76

Yang R, Brice B, Elliot A, Ryan U (2015) *Isospora serinuse* n. sp. (Apicomplexa: Eimeriidae) from a domestic canary (*Serinus canaria* forma domestica) (Passeriformes: Fringillidae) in Western Australia. Exp Parasitol 159:59–66

Yang R, Brice B, Liu Q, Berto BP, Austen J, Ryan U (2021) Morphological and genetic characterization of the first *Isospora* species (*I. lugensae* n. sp.) from a Kerguelen petrel (*Lugensa brevirostris*). Parasitol Res 120:1037–1047

Syst Parasitol (2022) 99:525–534
https://doi.org/10.1007/s11230-022-10042-8

Isospora leptopogoni n. sp. (Apicomplexa: Eimeriidae) from the sepia-capped flycatcher *Leptopogon amaurocephalus* Tschudi, 1846 (Passeriformes: Rhynchocyclidae) in South America

Jennifer Oliveira Melo · Lucas de Assis Silva Andrade · Carla Maronezi · Ericson Ramos de Mello · Mariana de Souza Oliveira · Sergian Vianna Cardozo · Heider Alves Franco · Águida Aparecida de Oliveira · Viviane Moreira de Lima · Bruno Pereira Berto

Received: 18 February 2022 / Accepted: 11 April 2022 / Published online: 14 May 2022

Abstract Coccidian protozoan species recorded from flycatchers are few, but they have been described with a certain frequency in recent years. In this context, the present study describes a new *Isospora* sp. from sepia-capped flycatchers *Leptopogon amaurocephalus* Tschudi, 1846 captured in the Itatiaia National Park and in a reforestation area which is about 60 km away from the park boundaries, in addition to providing a molecular identification via sequencing of the mitochondrial cytochrome *c* oxidase subunit 1 gene. *Isospora leptopogoni* **n. sp.** has oöcysts that are subspheroidal to ovoidal, measuring on average 22.0 × 19.7 µm, with a smooth, bi-layered wall, *c*.1.7 µm thick. The micropyle is delicate or inconspicuous. Oöcyst residuum is absent, but one to three polar granules are present. Sporocysts are lemon-shaped, measuring on average 14.7 × 9.3 µm, with a knob-like Stieda body and a rectangular to rounded

J. O. Melo
Curso de Graduação em Medicina Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7, Seropédica, Rio de Janeiro 23897-000, Brazil

L. A. S. Andrade · M. S. Oliveira
Programa de Pós-Graduação em Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7, Seropédica, Rio de Janeiro 23897-000, Brazil

C. Maronezi
Programa de Pós-Graduação em Ciência, Tecnologia e Inovação em Agropecuária, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7, Seropédica, Rio de Janeiro 23897-000, Brazil

E. R. de Mello
Programa de Pós-Graduação em Ciências Veterinárias, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7, Seropédica, Rio de Janeiro 23897-000, Brazil

S. V. Cardozo
Programa de Pós-Graduação em Biomedicina Translacional, Universidade do Grande Rio, Rua Professor José de Souza Herdy 1160, Duque de Caxias, Rio de Janeiro 25071-202, Brazil

H. A. Franco
Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro, Campus Pinheiral, Rua José Breves 550, Pinheiral, Rio de Janeiro 27197-000, Brazil

Á. A. de Oliveira
Departamento de Microbiologia e Imunologia Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7, Seropédica, Rio de Janeiro 23897-000, Brazil

V. M. de Lima · B. P. Berto (✉)
Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7, Seropédica, Rio de Janeiro 23897-000, Brazil
e-mail: bertobp@ufrrj.br

sub-Stieda body. Sporocyst residuum is present, consisting of compactly bounded granules. Sporozoites are vermiform, with refractile bodies and nucleus. *Isospora leptopogoni* is different from other *Isospora* spp. mainly due to its lemon-shaped sporocysts, the presence of micropyle and details of Stieda and sub-Stieda bodies. Phylogenetic analysis placed *I. leptopogoni* close to other *Isospora* spp. recorded from phylogenetically related hosts and from the same biogeographic region. Finally, the recurrent finding of this coccidian species in the same *L. amaurocephalus* specimen in a specific locality in the Itatiaia National Park suggests that the dispersion of *I. leptopogoni* needs continuous transmissions between susceptible passerines as the area of movement of each *L. amaurocephalus* specimen appears to be quite small.

Introduction

Parvorder Tyrannida comprises 320 species of passerines distributed in 10 different families in Brazil: Pipridae, Cotingidae, Tityridae, Oxyruncidae, Onychorhynchidae, Pipritidae, Platyrinchidae, Tachurisidae, Rhynchocyclidae and Tyrannidae (Pacheco et al., 2021). In addition to Brazil, the geographic distribution of this parvorder extends throughout the Americas, however, they are more predominant in the Neotropical Region (BirdLife International, 2022).

The sepia-capped flycatcher *Leptopogon amaurocephalus* Tschudi is a rhynchocyclid passerine commonly named as 'cabeçudo' in Brazil (Pacheco et al., 2021). This species occurs in almost the entire Neotropical region, on both *cis-* and *trans*-Andean sides. In Brazil it is mainly observed in the Atlantic Forest biome, in the South and Southeast regions (BirdLife International, 2022). It lives alone or in pairs, perching on vines and thin branches in exposed areas of the inner part of the forest (Sick, 1997). Although it often lives far from the ground and is an insectivorous bird, therefore being less predisposed to enteroparasites of faecal-oral transmission (Dolnik et al., 2010), there is a report of enteroparasitism by coccidian protozoa (Lopes et al., 2013).

Coccidians comprise many genera and species of parasites of domestic and wild animals, causing morbidity and mortality mainly in conditions of imbalance between host and environment (Berto & Lopes, 2020). From Tyrannida some coccidian species are recorded from the families Cotingidae, Tityridae, Platyrinchidae, Rhynchocyclidae and Tyrannidae. In this context, the present study provides a description and molecular identification of a new species of *Isospora* from sepia-capped flycatchers *L. amaurocephalus* captured in different localities in the Médio Paraíba region of the State of Rio de Janeiro, Southeastern Brazil.

Materials and methods

Sample collection

A total of nine expeditions were conducted in two different localities in the Médio Paraíba Region in the State of the Rio de Janeiro, Southeastern Brazil, which are about 60 km apart from each other: (1) Itatiaia National Park, a Brazilian federal conservation unit with a high degree of vulnerability, located in the Serra da Mantiqueira on the border of the States of Rio de Janeiro, Minas Gerais and São Paulo (ICMBIO, 2022); and (2) a reforestation area of 37 ha within the *campus* Pinheiral of the Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro – IFRJ, which is identified as Espaço Ecológico Educativo – EEcoE (Educational Ecological Space), at the municipality of Pinheiral in the State of the Rio de Janeiro, Southeastern Brazil. The first eight expeditions were conducted on November (22°26′57″S, 44°36′25″W) and December (22°27′20″S, 44°36′28″W) 2014, March (22°27′40″S, 44°35′32″W) and April (22°27′52″S, 44°36′26″W) 2015, October (22°27′40″S, 44°35′32″W) 2016, June (22°27′4″S, 44°36′51″W) and November (22°26′57″S, 44°36′25″W) 2017, and August (22°26′57″S, 44°36′25″W) 2018, in the Itatiaia National Park. The last expedition was conducted on January (22°31′37″S, 43°59′45″W) 2019 in the EEcoE/IFRJ. Mist nets were used for the capture of the birds. Eleven sepia-capped flycatchers *L. amaurocephalus* in the Itatiaia National Park and one in the EEcoE/IFRJ were captured. The birds were specifically identified (Ridgely et al., 2015), photographed and banded with numbered metal rings provided by the Brazilian bird-ringing agency (Centro Nacional de Pesquisa e Conservação de Aves Silvestres – CEMAVE). Subsequently, the birds were kept in individual boxes lined with clean paper until

defecation, when they were released at the same place of capture. Each fresh droplet of faeces (*c.*0.1 g) from each individual bird was placed individually in a centrifuge tube with a potassium dichromate 2.5% ($K_2Cr_2O_7$) solution (Dolnik, 2006).

Morphological analyses

Samples were taken to the Laboratório de Biologia de Coccídios, Universidade Federal Rural do Rio de Janeiro (UFRRJ), where they were incubated in the centrifuge tubes, and regularly oxygenated by shaking, at room temperature (~25°C) for 7 days. Oöcysts were recovered by the Sheather's method and identified microscopically according Duszynski & Wilber (1997) and Berto et al. (2014). Morphological observations, line drawings, photomicrographs and measurements were made using an Olympus BX binocular microscope (Olympus Optical, Tokyo, Japan) equipped with a Eurekam 5.0 digital camera (BEL Photonics, Monza, Italy). Line drawings were edited using two software applications (Corel DRAW and Corel PHOTO-PAINT) from CorelDRAW® (Corel Draw Graphics Suite, Version 2020, Corel Corporation, Ontario, Canada). All measurements are in micrometres and are given as the range followed by the mean in parentheses.

Molecular analyses

An individual oöcyst from each sample was isolated from serial dilutions of the oöcysts in drops on a microscope slide using a sterile micropipette. The isolated oöcyst was resuspended in PBS and washed by centrifuging until the supernatant became clear (Dolnik et al., 2009). DNA was extracted from the oöcyst using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, São Paulo, Brazil) according to the manufacturer's instructions. Four freeze-thaw cycles were applied prior to DNA extraction in order to achieve complete lysis of the oöcysts. PCR amplification of a partial fragment of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene (*c.*250 bp) was carried out using nested PCR, as previously described by Dolnik et al. (2009) and Yang et al. (2015). The nested PCR amplicons were purified using the Qiagen MinElute PCR Purification (Qiagen, São Paulo, Brazil).

DNA sequence analyses

Nested PCR amplicons were sequenced using an ABI-Prism 3500 Genetic Analyzer (Applied Biosystems, Foster City, California). The newly generated sequence was compared to those for *Isospora* spp. available in the GenBank database using the Basic Local Alignment Search Tool (BLAST). The phylogenetic tree was constructed using the newly generated COI sequence aligned with *Isospora* spp. available on GenBank. Alignments, distance analyses and phylogenies were conducted using Clustal W (http://www.clustalw.genome.jp) in MEGA X (Kumar et al., 2018). Maximum likelihood (ML) and Neighbor-Joining (NJ) trees were constructed, and the distances computed using the Tamura-Nei method based on model selection using ModelTest in MEGA X. Bootstrap analyses were conducted using 1000 replicates to assess the reliability of inferred tree topologies.

Results

Nine of the eleven (84%) sepia-capped flycatchers *L. amaurocephalus* captured in the Itatiaia National Park were positive for coccidian oöcysts. The sepia-capped flycatcher captured in the EEcoE/IFRJ was also positive. These oöcysts have a distinct morphotype from those coccidian species recorded as parasites of Tyrannida. This material is described below.

Family Eimeriidae Minchin, 1903

Genus *Isospora* Schneider, 1881

Isospora leptopogoni Melo & Berto n. sp.

Type-host: Leptopogon amaurocephalus Tschudi (Passeriformes: Tyranni: Tyrannides: Tyrannida: Rhynchocyclidae: Pipromorphinae), sepia-capped flycatcher.

Type-locality: Parque Nacional do Itatiaia (22°27′20″S, 44°36′28″W), southeastern Brazil.

Other locality: Espaço Ecológico Educativo (22°31′37″S, 43°59′45″W), Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro, campus Pinheiral, southeastern Brazil.

Type-material: Photosyntypes, line drawing and oöcysts in 2.5% $K_2Cr_2O_7$ solution (Williams et al.,

2010) are deposited and available (http://r1.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under the repository number P-127/2022. Photographs of the type-host specimen (symbiotype) are deposited in the same collection.
Site in host: Unknown.
Prevalence: 84% (10 out of 12 birds examined).
Representative DNA sequence: One representative COI sequence was deposited in the GenBank database under the accession number OM568833.
ZooBank registration: To comply with the regulations set out in Article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN, 2012) details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:3E3A9902-B7AE-407E-89AA-8A4624080B49. The LSID for the new name *Isospora leptopogoni* Melo & Berto **n. sp.** is urn:lsid:zoobank.org:act:14DAD073-D779-4762-A92A-AB88F5D6D253.
Etymology: The specific name is derived from the genus name of the type-host.

Description (Figs. 1, 2A–F)

Oöcyst (n = 63) subspheroidal to ovoidal, 19–25 × 18–23 (22.0 × 19.7); length/width (L/W) ratio 1.0–1.3 (1.12). Wall bi-layered, 1.5–1.9 (1.7) thick, outer layer smooth, c.2/3 of total thickness. Micropyle delicate or inconspicuous, 2.3–5.0 (3.8) wide. Oöcyst residuum absent, but 1–3 polar granules are present. Sporocyst lemon-shaped, 12–17 × 8–11 (14.7 × 9.3); L/W ratio 1.3–1.8 (1.58). Stieda body present, knob-like, 0.9–1.3 high × 1.6–2.2 wide (1.1 × 1.8). Sub-Stieda body present, rectangular to rounded, 1.3–2.2 high × 1.4–2.8 wide (1.6 × 2.1). Para-Stieda body absent. Sporocyst residuum present, consisting of compactly bounded granules. 4.1–7.3 × 3.7–6.8 (5.6 × 5.1). Sporozoites vermiform, with anterior and posterior refractile bodies and a central nucleus.

Phylogenetic analysis

DNA amplification of the oöcysts of *I. leptopogoni* **n. sp.** showed a clear band of *c.*250 bp. Sequences of two individual oöcysts from two different samples/birds were obtained and were 100% identical to each other. Phylogenetic analysis included 25 sequences for *Isospora* spp. available on GenBank (Fig. 3). *Toxoplasma gondii* (Nicolle & Manceaux, 1908) was used as the outgroup. *Isospora leptopogoni* **n. sp.** was recovered within a small monophyletic group with *Isospora borbai* Silva-Carvalho & Berto, 2019, *Isospora lopesi* Silva-Carvalho & Berto, 2018 and *Isospora sagittulae* McQuistion and Capparella, 1992, which are also parasites of suboscines passerines (Tyranni) from the Neotropical Region (Silva-Carvalho et al., 2018a, 2018b, 2019). This group of these three coccidians from suboscines was included within a larger clade with *Isospora massardi* Lopes, Berto, Luz, Galvão, Ferreira & Lopes, 2014, *Isospora sporophilae* Carvalho-Filho, Meireles, Ribeiro & Lopes, 2005, *Isospora sepetibensis* Berto, Flausino, Luz, Ferreira & Lopes, 2008 and *Isospora coerebae* Berto, Flausino, Luz, Ferreira & Lopes, 2010, which are also parasites of passerines from the Neotropical Region, although these are oscines passerines

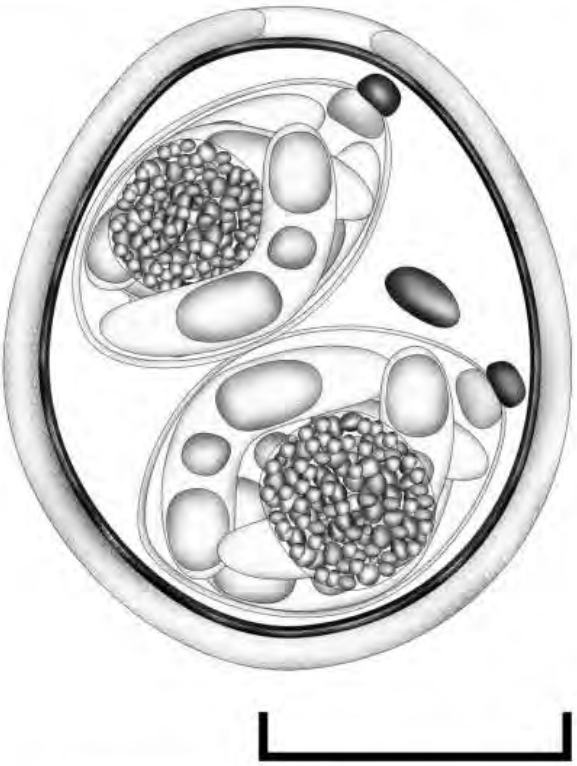

Fig. 1 Composite line drawing of the sporulated oöcyst of *Isospora leptopogoni* from sepia-capped flycatchers in South America. Scale-bar: 10 µm.

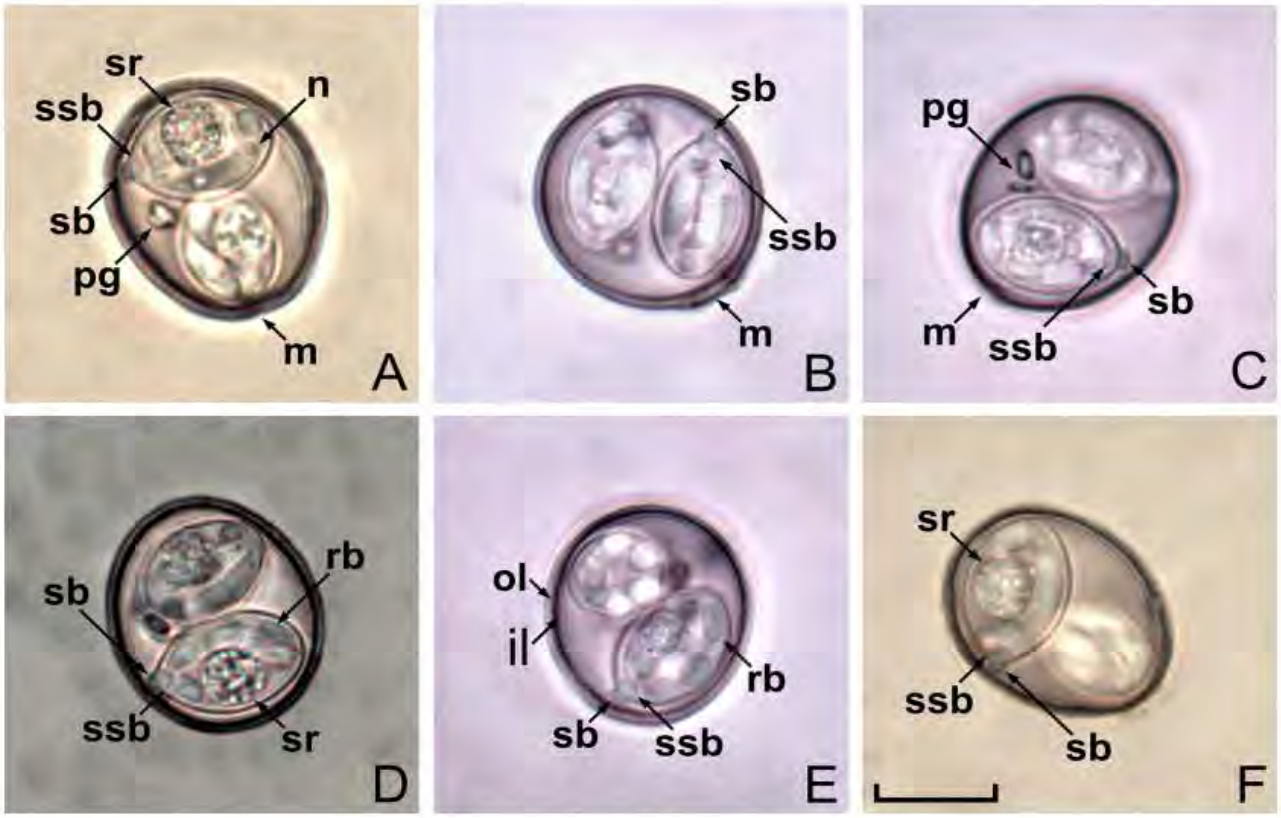

Fig. 2 Photomicrographs of *Isospora leptopogoni* recovered from sepia-capped flycatchers in South America. Note the inner (il) and the smooth outer (sol) layer of the oöcyst wall, micropyle (m), nucleous (n), polar granule (pg), refractile body (rb), sporocyst residuum (sr), and Stieda body (sb) and sub-stieda (ssb) bodies. Scale-bar: 10 μm.

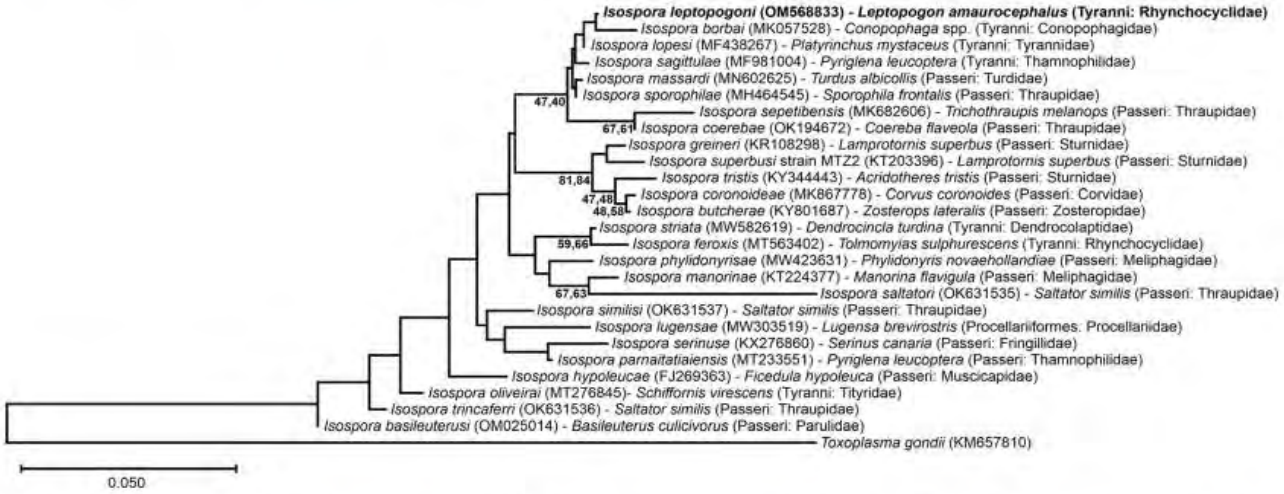

Fig. 3 Neighbor-Joining tree estimated from the COI gene sequences of *Isospora* species. Numbers at nodes represent bootstrap support 1000 replicates (> 40%) for Neighbor-Joining (NJ) and Maximum Likelihood (ML) respectively. Scale bar represents the number of nucleotide substitutions per site.

(Rodrigues et al., 2019, Genovez-Oliveira et al., 2019, 2020). All these *Isospora* spp. had the highest similarity of 99% with *I. leptopogoni*.

Discussion

Since the taxonomic review of coccidia of New World passerine birds by Berto et al. (2011), which is based on the guidelines of Duszynski & Wilber (1997), reports and descriptions of passerine coccidia have been studied and recorded according to the passerine host family. However, there is currently an inconsistency in the taxonomy of Aves when comparing different bibliographic sources, even if updated. In this case, BirdLife International (2022) classifies *L. amaurocephalus* as Tyrannidae, while the Brazilian Ornithological Records Committee (Pacheco et al., 2021) considers several smaller families organized into parvorders, superfamilies and subfamilies, derived from the large traditional family Tyrannidae. Because the Brazilian Ornithological Records Committee is the main bibliographic source of taxonomy of Aves in Brazil and establishes more classification groups facilitating the delimitation of phylogenetically closer and distant passerine hosts, this Brazilian classification was based for this study.

From Rhynchocyclidae, which is the family of *L. amaurocephalus*, only two coccidian species are recorded; therefore, this study compared the oöcysts obtained from *L. amaurocephalus* with *Isospora* spp. recorded from passerines of parvorder Tyrannida. Of the ten families that Tyrannida brings together, only five have coccidian species recorded, as shown in Table 1. *Isospora leptopogoni* can be easily distinguished from the six *Isospora* spp. recorded from Tyrannida by the lemon-shaped sporocyst with knob-like Stieda and rectangular to rounded sub-Stieda. Furthermore, it is the only one of these *Isospora* spp. to have smooth oöcysts with a delicate or inconspicuous micropyle (Table 1).

A specimen of *L. amaurocephalus* captured in November 2017 on a trail named 'Trilha das Borboletas' (Trail of the Butterflies) (22°26′57″S, 44°36′25″W) in Itatiaia National Park, was recaptured nine months later, in August 2018, in the same locality. This sepia-capped flycatcher has been ringed with the number ring 'C114236' (CEMAVE). Both samples collected in 2017 and 2018 were positive for *I. leptopogoni*, but with low densities of 81 and 74 oöcysts per faecal drop, respectively (Dolnik, 2006). This finding suggests that *L. amaurocephalus* specimens do not move over long distances individually, being probably restricted to small areas of movement. In this thought, the dispersion of *I. leptopogoni* must depend on frequent transmissions between *L. amaurocephalus* specimens, or other susceptible host species that are distributed in the Médio Paraíba Region where this study was developed, since the dispersion of coccidia by a single specimen would be quite restricted to small areas. It is also worth mentioning that the low densities and the absence of clinical signs observed in this specimen 'C114236', at different times, is compatible with the good state of conservation of the Itatiaia National Park (Berto & Lopes, 2020).

The primer used to amplify and sequence the genic region of ~250 bp of COI in this study has been considered unsuitable for phylogenetic studies, although it is being reasonably appropriate for the delimitation of *Isospora* spp. of passerines until then (Genovez-Oliveira et al., 2020; Maronezi et al., 2022; Mello et al., 2022). That is, despite some sequences of *Isospora* spp. from passerines being 100% identical to each other for this same genic region when analyzed by BLAST, *I. leptopogoni* was not 100% identical with any sequence deposited in GenBank. In the phylogenetic analysis (Fig. 3) the sequence of *I. leptopogoni* sat close to coccidian species from phylogenetically close passerines and from the same biogeographic region; although other *Isospora* spp. also from phylogenetically related passerines, such as *Isospora feroxis* Berto, Luz, Flausino, Ferreira & Lopes, 2009a, 2009b, were distant in the phylogenetic analysis. Despite these phylogenetic observations, the support values for the tree were very low, including for the placement of *I. leptopogoni*, making any interpretation of the topology with little meaning. Anyway, this ~250 bp sequence of *I. leptopogoni*, even if short and phylogenetically inconclusive, represents a minimal molecular identification for this species that separates it from other *Isospora* spp. from passerines until then deposited in GenBank; in addition, this sequence may be used in the future to complement the sequencing of other nearby genic regions in their mitochondrial genome.

Finally, the comparison of *I. leptopogoni* with *Isospora* spp. recorded from Tyrannida birds (Table 1),

Table 1 Comparative morphology of *Isospora* spp. recorded from Tyrannida birds.

Species	Host	Reference	Oöcyst					
			Shape	Size (µm)	L/W ratio	Polar granule	Wall	Micropyle
Isospora araponga Doležalová, Torres, Fernández & Modrý, 2004	*Procnias nudicollis* (Vieillot) (Cotingidae: Cotinginae)	Doležalová et al. (2004)	subspheroidal to ellipsoidal	17–22 × 14–16 (19 × 15)	1.1–1.4 (1.3)	1–3	smooth	absent
Isospora feroxis Berto, Luz, Flausino, Ferreira & Lopes, 2009a, 2009b	*Myiarchus ferox* (Gmelin) (Tyrannidae: Tyranninae)	Berto et al. (2009a)	subspheroidal	18–20 × 17–20 (19 × 18)	1.0–1.1 (1.1)	usually 2	low roughness	absent[1]
	M. ferox (Tyrannidae: Tyranninae)	Ortúzar-Ferreira et al. (2021)	subspheroidal	21–23 × 20–23 (22 × 21)	1.0–1.1 (1.0)	1–3 (usually 2 bonded)	low to moderate roughness	present
	Tolmomyias sulphurescens Spix (Rhynchocyclidae: Rhynchocyclinae)			18–23 × 18–22 (20 × 20)	1.0–1.1 (1.0)			
Isospora mionectesi Berto, Flausino, Luz, Ferreira & Lopes, 2009a, 2009b	*Mionectes rufiventris* Cabanis (Rhynchocyclidae: Pipromorphinae)	Berto et al. (2009b)	ellipsoidal	26–31 × 19–23 (28 × 21)	1.2–1.4 (1.3)	usually 1-2	smooth	absent
Isospora attilae Rodrigues, Silva, Lopes, Berto, Luz, Ferreira & Lopes, 2015	*Attila rufus* (Vieillot) (Tyrannidae: Tyranninae)	Rodrigues et al. (2015)	subspheroidal to elipsoidal	18–22 × 18–21 (20 × 19)	1.0–1.2 (1.1)	1–2	smooth	absent
Isospora lopesi Silva-Carvalho & Berto, 2018	*Platyrinchus mystaceus* Vieillot (Platyrinchidae)	Silva-Carvalho et al. (2018a, 2018b)	subspheroidal to ovoidal	18–24 × 18–22 (21 × 20)	1.0–1.2 (1.1)	1	smooth	absent
Isospora oliveirai Ortúzar-Ferreira & Berto. 2020	*Schiffornis virescens* (Lafresnaye) (Tityridae: Schiffornithinae)	Ortúzar-Ferreira et al. (2020)	subspheroidal	24–28 × 23–27 (26 × 25)	1.0–1.1 (1.0)	1–6	slightly rough	absent
Isospora leptopogoni Melo & Berto **n. sp.**	*Leptopogon amaurocephalus* Tschudi (Rhynchocyclidae: Pipromorphinae)	present study	subspheroidal to ovoidal	19–25 × 18–23 (22 × 20)	1.0–1.3 (1.1)	1–3	smooth	present, delicate or inconspicuous

Table 1 continued

Species	Host	Reference	Sporocyst					
			Shape	Size (µm)	L/W ratio	Stieda body	Sub-Stieda body	Sporocyst residuum
Isospora araponga Doležalová, Torres, Fernández & Modrý, 2004	*Procnias nudicollis* (Vieillot) (Cotingidae: Cotinginae)	Doležalová et al. (2004)	ellipsoidal slightly asymmetric	12–13 × 7–9 (12 × 8)	1.3–1.7 (1.5)	barely visible	absent	compact
Isospora feroxis Berto, Luz, Flausino, Ferreira & Lopes, 2009a, 2009b	*Myiarchus ferox* (Gmelin) (Tyrannidae: Tyranninae)	Berto et al. (2009a)	ovoidal	11–13 × 8–10 (12 × 8)	1.0–1.5 (1.4)	flattened, (0.3 × 1.2)	prominent, (1.2 × 2.5)	diffuse
	M. ferox (Tyrannidae: Tyranninae)	Ortúzar-Ferreira et al. (2021)	ovoidal to ellipsoidal	14–15 × 9–10 (15 × 10)	1.4–1.6 (1.5)	flattened to half-moon-shaped, 0.5–0.7 × 1.3–2.2 (0.6 × 1.7)	rounded to trapezoidal, 1.0–1.8 × 2.4–3.3 (1.3 × 2.9)	diffuse
	Tolmomyias sulphurescens Spix (Rhynchocyclidae: Rhynchocyclinae)			11–15 × 8–10 (13 × 9)	1.3–1.6 (1.5)			
Isospora mionectesi Berto, Flausino, Luz, Ferreira & Lopes, 2009a, 2009b	*Mionectes rufiventris* Cabanis (Rhynchocyclidae: Pipromorphinae)	Berto et al. (2009b)	elongate-ellipsoidal	17–22 × 10–13 (20 × 12)	1.6–1.8 (1.7)	rounded, (0.8 × 1.1)	prominent, (1.4 × 2.1)	compact, subspherical
Isospora attilae Rodrigues, Silva, Lopes, Berto, Luz, Ferreira & Lopes, 2015	*Attila rufus* (Vieillot) (Tyrannidae: Tyranninae)	Rodrigues et al. (2015)	ellipsoidal	12–15 × 7–9 (13 × 8)	1.6–1.9 (1.7)	knob like, (1.0 × 2.0)	rounded to trapezoidal, (2.5 × 4.0)	diffuse
Isospora lopesi Silva-Carvalho & Berto, 2018	*Platyrinchus mystaceus* Vieillot (Platyrinchidae)	Silva-Carvalho et al. (2018a, 2018b)	ellipsoidal	12–16 × 8–11 (15 × 9)	1.5–1.9 (1.7)	flattened to half-moon-shaped, (1.0 × 2.5)	rounded, (2.0 × 2.5)	diffuse
Isospora oliveirai Ortúzar-Ferreira & Berto, 2020	*Schiffornis virescens* (Lafresnaye) (Tityridae: Schiffornithinae)	Ortúzar-Ferreira et al. (2020)	lemon-shaped	10–11 × 6–7 (10 × 6)	1.6–1.7 (1.6)	knob-like to half-moon-shaped, (2.0 × 3.5)	rounded, (2.5 × 3.5)	diffuse
Isospora leptopogoni Melo & Berto **n. sp.**	*Leptopogon amaurocephalus* Tschudi (Rhynchocyclidae: Pipromorphinae)	present study	lemon-shaped	12–17 × 8–11 (15 × 9)	1.3–1.8 (1.6)	knob-like, (1.1 × 1.8)	rectangular to rounded, (1.6 × 2.1)	compact

[1]The misidentification of the absence of micropyle was corrected for *E. feroxis* in the later work by Ortúzar-Ferreira et al. (2021).

in addition to molecular identification by sequencing the COI gene, clearly supports the designation as a unique species. Therefore, *I. leptopogoni* is considered as new to science, being the third species described from Rhynchocyclidae and the seventh recorded in the parvorder Tyrannida.

Acknowledgements We are thankful to staff at the Parque Nacional do Itatiaia, mainly to the research coordinators Dr Léo Nascimento (previous) and Marcelo Souza Motta (current); and the Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro, *Campus* Pinheiral; that allowed us to access and use some facilities during the expeditions.

Funding This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ). JOM has a scholarship from CNPq/UFRRJ (Grant/Award Number: PVBS2480-2021). LASA and CM have scholarships from CAPES (Grant/Award Number: 001). MSO has a post-doctoral scholarship from FAPERJ (Grant/Award Number: E-26/204.228/2021). BPB has fellowships from CNPq (Grant/Award Number: 303899/2019-0) and from FAPERJ (Grant/Award Number: E-26/202.797/2019).

Data availability All data generated or analyzed during this study are included in the article.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Field-collecting permits were issued by SISBIO/ICMBio (licenses 45200; 49605; 54951; 70132), CEUA/UFRRJ (protocols IV-036/2014; ICBS-008/2015; IV-6606250616) and CEUA/UNIGRANRIO (protocol 021/2019). Banding permits and metal rings were issued by CEMAVE/ICMBio (Senior Ringer: BPB, registration 5967850; Junior Ringer: MSO, registration 7035678). All applicable institutional, national and international guidelines for the care and use of animals were followed.

References

Berto, B. P., Luz, H. R., Flausino, W., Ferreira, I., & Lopes, C. W. G. (2009a). New species of *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) from the short-crested flycatcher *Myiarchus ferox* (Gmelin) (Passeriformes: Tyrannidae) in South America. *Systematic Parasitology, 74*, 75–80. https://doi.org/10.1007/s11230-009-9197-3

Berto, B. P., Flausino, W., Luz, H. R., Ferreira, I., & Lopes, C. W. G. (2009b). *Isospora mionectesi* sp. nov. (Apicomplexa, Eimeriidae) from the grey-hooded flycatcher, *Mionectes rufiventris* in Brazil. *Acta Parasitologica, 54*, 301–304. https://doi.org/10.2478/s11686-009-0053-z

Berto, B. P., Flausino, W., McIntosh, D., Teixeira-Filho, W. L., & Lopes, C. W. G. (2011). Coccidia of New World passerine birds (Aves: Passeriformes): a review of *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae). *Systematic Parasitology, 80*, 159–204. https://doi.org/10.1007/s11230-011-9317-8

Berto, B. P., McIntosh, D., & Lopes, C. W. G. (2014). Studies on coccidian oocysts (Apicomplexa: Eucoccidiorida). *Revista Brasileira de Parasitologia Veterinária, 23*, 1–15. https://doi.org/10.1590/S1984-29612014001

Berto, B. P., & Lopes, C. W. G. (2020). Coccidia of wild birds as ecological biomarkers: Some approaches on parasite-host-environment interaction. *Journal of Parasitology, 106*, 707–713. https://doi.org/10.1645/19-148

BirdLife International. (2022). IUCN Red List for birds. http://www.birdlife.org. Accessed 26 Jan 2022.

Dolezalová, M., Torres, J., Fernández, H., & Modrý, D. (2004). *Isospora araponga* sp. n. (Apicomplexa: Eimeriidae), a new species of *Isospora* Schneider from a bare-throated bellbird, *Procnias nudicollis* (Vieillot, 1817) (Passeriformes: Cotingidae) from Brazil. *Memórias do Instituto Oswaldo Cruz, 99*, 829–830. https://doi.org/10.1590/S0074-02762004000800008

Dolnik, O. (2006). The relative stability of chronic *Isospora sylvianthina* (Protozoa: Apicomplexa) infection in blackcaps (*Sylvia atricapilla*): evaluation of a simplified method of estimating isosporan infection intensity in passerine birds. *Parasitology Research*, 100, 155–160. https://doi.org/10.1007/s00436-006-0253-5

Dolnik, O. V., Palinauskas, V., & Bensch, S. (2009). Individual oocysts of *Isospora* (Apicomplexa: Coccidia) parasites from avian feces: from photo to sequence. *Journal of Parasitology, 95*, 169–174. https://doi.org/10.1645/GE-1873.1

Dolnik, O.V., Dolnik, V.R., & Bairlein, F. (2010). The effect of host foraging ecology on the prevalence and intensity of coccidian infection in wild passerine birds. Ardea 98, 97–103. https://doi.org/10.5253/078.098.0112

Duszynski, D. W., & Wilber, P. G. (1997). A guideline for the preparation of species descriptions in the Eimeriidae. *Journal of Parasitology, 83*, 333–336. https://doi.org/10.2307/3284470

Genovez-Oliveira, J.L., Cardozo, S.V., Oliveira, Á.A., Lima, V.M., Ferreira, I., & Berto, B.P. (2019). Morphological and molecular identification of *Isospora sepetibensis* (Chromista: Miozoa: Eimeriidae) from a new host, *Trichothraupis melanops* (Passeriformes: Thraupidae: Tachyphoninae) in South America. *Acta Protozoologica*, 58, 17–23. https://doi.org/10.4467/16890027AP.19.007.10838

Genovez-Oliveira, J.L., Oliveira, M.S., Thode-Filho, S., Cardozo, S.V., Oliveira, Á.A., Lima, V.M., Ferreira, I., & Berto, B.P. (2020). Morphological and molecular identification of *Isospora massardi* Lopes, Berto, Luz, Galvão, Ferreira & Lopes, 2014 (Chromista: Miozoa: Eimeriidae) from thrushes *Turdus* spp. (Passeriformes: Turdidae) in South America. *Parasitology International, 75*, 102040. https://doi.org/10.1016/j.parint.2019.102040

ICMBIO. (2022). Parque Nacional do Itatiaia. http://www.icmbio.gov.br/parnaitatiaia. Accessed 26 Jan 2022.

ICZN. (2012). International Commission on Zoological Nomenclature: Amendment of Articles 8, 9, 10, 21 and 78 of the International Code of Zoological Nomenclature to expand and refine methods of publication. *Bulletin of zoological nomenclature, 69*, 161–169. https://doi.org/10.21805/bzn.v69i3.a8.161

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution, 35*, 1547–1549. https://doi.org/10.1093/molbev/msy096

Lopes, B.D., Berto, B.P., Ferreira, I., Luz, H.R., & Lopes, C.W.G. (2013). Coccidial distribution from passerines in an area of Atlantic Forest in Marambaia Island, Rio de Janeiro, Brazil. *Coccidia, 1*, 10–16.

Maronezi, C., Oliveira, M.S., Genovez-Oliveira, J.L., Mello, E.R., Cepeda, P.B., Oliveira, Á.A., Lima, V.M., & Berto, B.P. (2022) *Isospora* spp. (Eimeriidae) from green-winged saltators *Saltator similis* d'Orbigny & Lafresnaye, 1837 (Thraupidae) from captivity near the Conservation Unit of the Itatiaia National Park in Southeastern Brazil. *Systematic Parasitology, 99*, 285–297. https://doi.org/10.1007/s11230-022-10025-9

Mello, E.R., Oliveira, M.S., Andrade, L.A.S., Cardozo, S.V., Oliveira, Á.A., Lima, V.M., & Berto, B.P. (2022). *Isospora basileuterusi* n. sp. (Apicomplexa: Eimeriidae) from the golden-crowned warbler *Basileuterus culicivorus* (Deppe) (Passeriformes: Parulidae) in South America. *Current Research in Parasitology & Vector-Borne Diseases, 2*, 100079. https://doi.org/10.1016/j.crpvbd.2022.100079

Ortúzar-Ferreira, C.N., Genovez-Oliveira, J.L., Oliveira, M.S., Mello, E.R., Thode-Filho, S., Oliveira, Á.A., Lima, V.M., Ferreira, I., & Berto, B.P. (2020). *Isospora oliveirai* n. sp. (Chromista: Miozoa: Eimeriidae) from the greenish schiffornis *Schiffornis virescens* (Lafresnaye, 1838) (Passeriformes: Tyranni: Tityridae) in South America. *Acta Parasitologica, 65*, 843–851. https://doi.org/10.1007/s11686-020-00237-8

Ortúzar-Ferreira, C.N., Mello, E.R., Melo, J.O., Oliveira, M.S., Thode-Filho, S., Cardozo, S.V., Oliveira, Á.A., Lima, V.M., Ferreira, I., Berto, B.P. (2021). Redescription and molecular identification of *Isospora feroxis* Berto, Luz, Flausino, Ferreira & Lopes, 2009 (Eimeriidae) from tyrant-flycatchers (Tyrannoidea) in South America. *Systematic Parasitology, 98*, 333–341. https://doi.org/10.1007/s11230-021-09981-5

Pacheco, J.F., Silveira, L.F., Aleixo, A., Agne, C.E., Bencke, G.A., Bravo, G.A., Brito, G.R.R., Cohn-Haft, M., Maurício, G.N., Naka, L.N., Olmos, F., Posso, S., Lees, A.C., Figueiredo, L.F.A., Carrano, E., Guedes, R.C., Cesari, E., Franz, I., Schunck, F., & Piacentini, V.Q. (2021). Annotated checklist of the birds of Brazil by the Brazilian Ornithological Records Committee – second edition. *Ornithology Research, 29*, 94–105. https://doi.org/10.1007/s43388-021-00058-x

Ridgely, R.S., Gwynne, J.A., Tudor, G., & Argel, M. (2015). *Aves do Brasil: Mata Atlântica do Sudeste*. São Paulo: Horizonte, 417 pp.

Rodrigues, M. B., Silva, L. M., Lopes, B. do B., Berto, B. P., Luz, H. R., Ferreira, I., & Lopes, C. W. G. (2015). A new species of *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae) from the grey-hooded attila *Attila rufus* Vieillot, 1819 (Passeriformes: Tyrannidae) on the Marambaia Island, Brazil. *Zootaxa, 4034*, 193–196. https://doi.org/10.11646/zootaxa.4034.1.10

Rodrigues, M.B., Oliveira, J.L.G., Silva-Carvalho, L.M., Pastura, D.G.N., Gomes, J.V., Oliveira, M.S., Siqueira, P.B., Oliveira, Á.A., Lima, V.M., Ferreira, I., Berto, B.P. (2019). The vulnerable *Sporophila frontalis* (Verreaux) and *Haplospiza unicolor* Cabanis as new hosts for *Isospora sporophilae* Carvalho-Filho, Meireles, Ribeiro & Lopes, 2005 (Eimeriidae) in Brazil. *Systematic Parasitology, 96*, 423-431. https://doi.org/10.1007/s11230-019-09859-7

Sick, H. (1997). *Ornitologia Brasileira*. Rio de Janeiro: Nova Fronteira, 862 pp.

Silva-Carvalho, L. M., Pastura, D. G. N., Gomes, J. V., Siqueira, P. B., Rodrigues, M. B., Lima, V. M., & Berto, B. P. (2018a). *Isospora lopesi* n.sp. (Apicomplexa: Eimeriidae) from the eastern white-throated spadebill *Platyrinchus mystaceus* Vieillot (Passeriformes: Tyranni: Tyrannidae) in South America. *Systematic Parasitology, 95*, 455–463. https://doi.org/10.1007/s11230-018-9795-z

Silva-Carvalho, L.M., Pastura, D.G., Rodrigues, M.B., Gomes, J.V., Oliveira, M.S., Siqueira, P.B., Genovez-Oliveira, J.L., Soares, S.S., Oliveira, Á.A., Lima, V.M., Ferreira, I., Berto, B.P. (2018b). *Isospora sagittulae* McQuistion & Capparella, 1992 (Apicomplexa: Eimeriidae) from antbirds (Passeriformes: Thamnophilidae) in the Amazon and Atlantic Forest of Brazil: with notes on its distribution and dispersion in the Neotropical region. *Parasitology Research, 117*, 2635–2641. https://doi.org/10.1007/s00436-018-5955-y

Silva-Carvalho, L.M., Genovez-Oliveira, J.L., Oliveira, M.S., Rodrigues, M.B., Abreu, S.T., Ortúzar-Ferreira, C.N., Franco, H.A., Thode-Filho, S., Oliveira, Á.A., Lima, V.M., Ferreira, I., Berto, B.P. (2019). *Isospora borbai* n. sp. (Chromista: Apicomplexa: Eimeriidae) from gnateaters *Conopophaga* spp.(Passeriformes: Tyranni: Conopophagidae) in South America. *Acta Parasitologica, 64*, 617–624. https://doi.org/10.2478/s11686-019-00079-z

Williams, R. B., Thebo, P., Marshall, R. N., & Marshall, J.A. (2010). Coccidian oöcysts as type-specimens: long-term storage in aqueous potassium dichromate solution preserves DNA. *Systematic Parasitology, 76*, 69–76. https://doi.org/10.1007/s11230-010-9234-2

Yang, R., Brice, B., Elliot, A., & Ryan, U. (2015). *Isospora serinuse* n. sp. (Apicomplexa: Eimeriidae) from a domestic canary (*Serinus canaria forma domestica*) (Passeriformes: Fringillidae) in Western Australia. *Experimental Parasitology, 159*, 59–66. https://doi.org/10.1016/j.exppara.2015.08.020

Acta Parasitologica (2022) 67:1343–1353
https://doi.org/10.1007/s11686-022-00585-7

ORIGINAL PAPER

Worldwide Dispersion of Coccidia from Migratory Birds: First Report of *Eimeria bazi* Chauhan et Bhatia, 1970 (Eimeriidae) Outside Asia from Buff-Necked Ibises *Theristicus caudatus* (Boddaert, 1783) (Threskiornithidae) in South America

Rísia Brígida Gonçalves Cabral[1] · Carlos Nei Ortúzar-Ferreira[2] · Mariana de Souza Oliveira[1] · Ericson Ramos de Mello[3] · Águida Aparecida de Oliveira[4] · Viviane Moreira de Lima[5] · Ildemar Ferreira[5] · Bruno Pereira Berto[5]

Received: 10 March 2022 / Accepted: 15 June 2022 / Published online: 14 July 2022

Abstract

Background *Eimeria* spp. are coccidian protozoan parasites of domestic and wild animals. Pelecaniform birds are hosts of some *Eimeria* spp., however, from the family Threskiornithidae only one eimerian species is recorded, *Eimeria bazi* Chauhan et Bhatia, 1970 which was described from red-naped ibises *Pseudibis papillosa* (Temminck, 1824) in India. In this study, in turn, this species is morphologically and molecularly identified from buff-necked ibises *Theristicus caudatus* (Boddaert, 1783) in Brazil.

Purpose This study aimed to report *E. bazi* from buff-necked ibises *T. caudatus* in southeastern Brazil, revealing the worldwide distribution of this coccidian species, in addition to providing preliminary genotypic identification via sequencing of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene.

Methods A total of 73 fecal samples were collected from a flock of buff-necked ibises, which remained on the campus of the Federal Rural University of Rio de Janeiro (Universidade Federal Rural do Rio de Janeiro—UFRRJ) from March 2019 to August 2020. Fecal samples were processed by the Sheather's method to recover oocysts. The morphological and morphometrical studies of the oocysts were performed using an optical microscope and graphic editing software. Molecular analysis was performed by sequencing of the COI gene, and the phylogenetic analysis was based in the neighbor-joining and maximum likelihood estimates.

Results Forty-five fecal samples were positive for oocysts identified as *E. bazi*. This oocysts are ovoidal, 26.2 × 18.9 µm, with smooth to slightly rough wall, *c.*1.7 µm thick. Micropyle robust and protruding, sometimes with a polar body attached. Oocyst residuum absent, but one or two small polar granules are present. Sporocysts ovoidal to lemon-shaped, 14.2 × 8.7 µm. The Stieda body is knob-like to rounded and sub-Stieda body is absent or vestigial. Sporocyst residuum is composed of granules often membrane-bound. Sporozoites are vermiform, with refractile bodies. This morphology was consistent with the original description of *E. bazi* from *P. papillosa* in India. Molecular analysis at the COI gene exhibited low similarity with coccidians sequenced for the same genic region deposited in GenBank, sitting *E. bazi* separately on the cladogram.

Conclusions The morphological and molecular studies support the identification of *E. bazi* from *T. caudatus* in South America, thus revealing the wide distribution of this eimerian species in the world provided by migratory birds and/or with intercontinental distribution.

Keywords Morphology · Molecular biology · Taxonomy · Phylogeny · Coccidia · Oocysts · Migratory birds · Ibises · Federal Rural University of Rio de Janeiro

✉ Bruno Pereira Berto
bertobp@ufrrj.br

Extended author information available on the last page of the article

Introduction

Coccidia are a highly diverse subclass of protozoan obligate intracellular parasites of domestic and wild animals [1, 2]. *Eimeria* Schneider, 1875 is the genus most predominant and diverse, being identified from mammals, birds, reptiles, amphibians and fish, in addition to invertebrates [3]. From birds, the most prevalent coccidian genera are *Isospora* Schneider, 1881 and *Eimeria*; however, *Isospora* spp. occur more frequently in passeriform birds, while *Eimeria* spp. are more frequent in non-passeriform birds [3].

The buff-necked ibis *Theristicus caudatus* (Boddaert, 1783) is a non-passeriform bird of the order Pelecaniformes, family Threskiornithidae. This species is among the ibises with the widest geographic distribution in the Neotropical Region [4]. It is a bird typical of grasslands and occurs in a wide variety of open and semi-open habitats, such as grasslands and savannas, in addition to lawns on the shores of lakes, ponds and other watercourses [5]. It forages in flocks, but also occasionally solitary, in soft soils and grasslands. It feeds on small reptiles, amphibians and arthropods, mainly insects including their larvae, which are captured directly from the substrate and through the deep penetration of its long beak into the soil [6]. It is a mainly sedentary species with predominance of local movements; however, it has expanded its geographic distribution, especially in southeastern Brazil [7, 8].

The habits of the buff-necked ibises living in flocks, foraging in contact with the ground and having a predominance of local movements, favor the biology of coccidian parasites [9]. In general, infected birds shed coccidian oocysts into the environment when defecating, which will become sporulated/viable depending on the conditions of humidity, temperature and atmospheric oxygen [2]. Then, the habits of the buff-necked ibises would ensure the continuity of the life cycle of its coccidians, since the presence of other susceptible ibises in the same environment would be constant [9].

Despite this favorable context for coccidiosis in buff-necked ibises, there are no descriptions of coccidian species parasitizing *T. caudatus*; however, there is one eimerian species recorded from Threskiornithidae, *Eimeria bazi* Chauhan et Bhatia, 1970 described from red-naped ibises *Pseudibis papillosa* (Temminck, 1824) in India [10]. Consequently, in this study, fecal samples were collected from a flock of buff-necked ibises *T. caudatus* that remained on the campus of the Federal Rural University of Rio de Janeiro (Universidade Federal Rural do Rio de Janeiro—UFRRJ) from March 2019 to August 2020, aiming to recover, isolate and identify coccidian oocysts.

Materials and Methods

Sample Collection

Eight fieldworks were carried out to collect fecal samples from the flock of buff-necked ibises *T. caudatus* that remained on the Seropédica campus of UFRRJ (22° 45′ 46″ S, 43° 41′ 18″ W), from March 2019 to August 2020. The monospecific flock of ibises were observed from a distance until defecations were seen, which were then sought and found. Depending on the type of surface that the feces were found, they were discarded, giving preference to those droplets of feces shed on leaves, rocks, or other surfaces less susceptible to contamination. Each fresh droplet of feces from each individual ibis was placed individually in a centrifuge tube with a potassium dichromate 2.5% ($K_2Cr_2O_7$) solution at 1:6 (v/v).

Morphological Analyses

Fecal samples were transported to the Laboratório de Biologia de Coccídios, Universidade Federal Rural do Rio de Janeiro (UFRRJ). In laboratory, the samples were kept at room temperature (~25 °C) for 7 days in the same centrifuge tubes which were collected, being oxygenated daily by shaking until the oocysts were sporulated. Oocysts were recovered and isolated using the Sheather's method and were morphologically studied following the guidelines of Duszynski and Wilber [11] and Berto *et al.* [12]. Optical microscopy, photomicrography and morphometry were performed using an Olympus BX binocular microscope (Olympus Optical, Tokyo, Japan) equipped with a digital camera Eurekam 5.0 (BEL Photonics, Monza, Italy). Line drawings were performed using the applications Corel DRAW and Corel PHOTO-PAINT from CorelDRAW® Graphics Suite (Version 2020, Corel Corporation, Canada). Measurements (in micrometers) are given as the range followed by the mean in parentheses.

Molecular Analyses

A single oocyst from each sample was individualized from serial dilutions of the oocysts in drops on a microscope slide using an automatic micropipette. The individualized oocyst was transferred to a microtube containing PBS and washed by centrifuging until the solution is clear [13]. Four freeze–thaw cycles were performed to achieve complete lysis of the oocyst. DNA extraction was performed using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, São Paulo, Brazil). Amplification by PCR of a partial fragment of the mitochondrial cytochrome *c* oxidase subunit 1 (COI)

gene (*c.*250 bp) was performed using nested PCR, according Dolnik *et al.* [13] and Yang *et al.* [14]. The nested PCR amplicons were purified using the Qiagen MinElute PCR Purification (Qiagen, São Paulo, Brazil).

DNA Sequence Analyses

Nested PCR amplicons were sequenced using an ABI-Prism 3500 Genetic Analyzer (Applied Biosystems, Foster City, California). The newly generated sequence was compared to those for coccidian species available in the GenBank database using the Basic Local Alignment Search Tool (BLAST). The phylogenetic tree was constructed using the newly generated COI sequence aligned with coccidian species available on GenBank. Distance analyses and phylogenies were conducted using MEGA X [15]. Briefly, the nucleotide sequences were imported into MEGA X and aligned with reference sequences from GenBank using Clustal W (http://www.clustalw.genome.jp). Maximum likelihood (ML) and neighbor-joining (NJ) trees were constructed, and the distances computed using the Tamura-Nei method based on model selection using ModelTest in MEGA X. Bootstrap analyses were conducted using 1000 replicates to assess the reliability of inferred tree topologies.

Results

A total of 73 fecal samples were collected from buff-necked ibises and 45 were positive for coccidia. All observed oocysts were morphologically identified as *E. bazi*. This material is described below.

Family Eimeriidae Minchin, 1903

Genus *Eimeria* Schneider, 1875

***Eimeria bazi* Chauhan et Bhatia, 1970** (Figs. 1, 2)

Oocysts ($n=88$) ovoidal, 21–30×16–21 (26.2×18.9); length/width (L/W) ratio 1.2–1.6 (1.39). Wall bi-layered, 1.5–1.9 (1.7) thick, outer layer smooth to slightly rough, *c.*2/3 of total thickness. Micropyle present, robust and protruding, 4.1–5.4 (4.8) wide; occasionally with a rounded polar body attached to the micropyle, 1.6–2.3×1.5–2.1 (2.0×1.7). Oocyst residuum absent, but one or two small polar granules are present. Sporocysts (n=30) ovoidal to lemon-shaped, 13–15×7–10 (14.2×8.7); L/W ratio 1.4–1.8 (1.65). Stieda body present, knob-like to rounded, 0.7–1.4×1.3–1.9 (1.1×1.5); sub-Stieda body absent or vestigial; para-Stieda body absent; sporocyst residuum present, composed of granules often membrane-bound in the center of the sporocyst, 3.4–5.5×3.5–4.3 (4.7×4.0), but also scattered among the sporozoites. Sporozoites vermiform, with anterior and posterior refractile bodies, but nucleus is indiscernible.

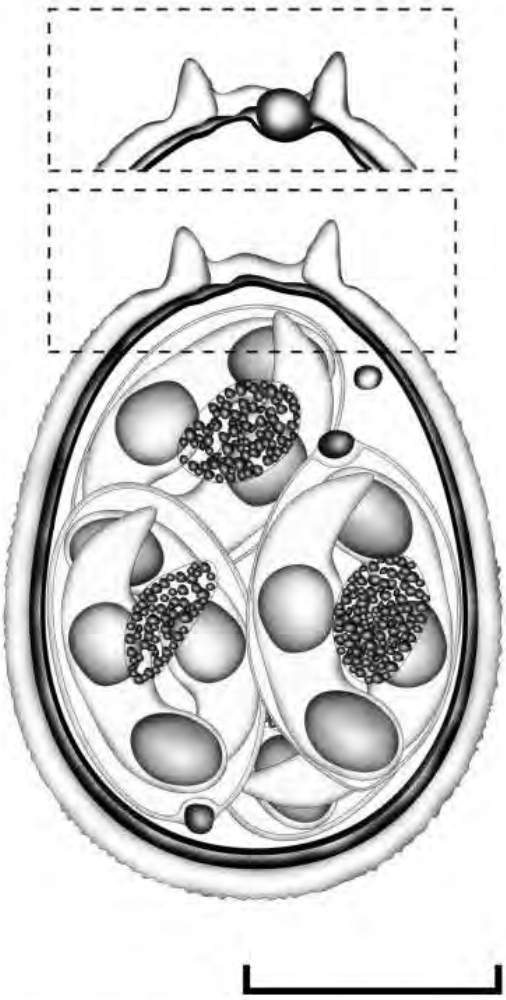

Fig. 1 Composite line drawing of the sporulated oocyst of *Eimeria bazi* from the buff-necked ibis *Theristicus caudatus*, highlighting the micropyle with and without an attached polar body. Scale-bar: 10 µm

Host: *Theristicus caudatus* (Boddaert, 1783) (Aves: Pelecaniformes: Threskiornithidae), buff-necked ibis.

Locality: *Campus* of the Federal Rural University of Rio de Janeiro (22° 45′ 46″ S, 43° 41′ 18″ W), southeastern Brazil.

Representative specimens: Photomicrographs, line drawing and oocysts in 2.5% $K_2Cr_2O_7$ solution (Williams *et al.* 2010) are deposited and available (http://r1.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under the repository number 128/2022. Photographs of the host specimen are deposited in the same collection.

Site in host: Unknown.

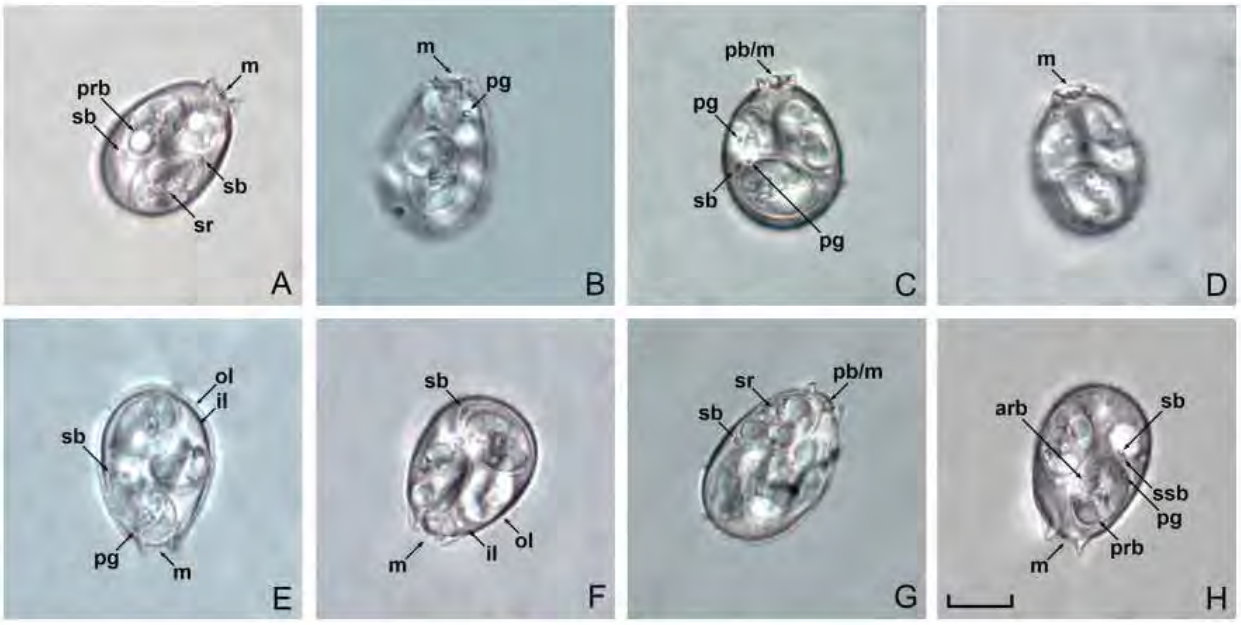

Fig. 2 Photomicrographs of sporulated oocysts of *Eimeria bazi* from the buff-necked ibis *Theristicus caudatus*. inner (il) and outer (ol) layers of the oocyst wall; micropyle (m) and polar body attached to the micropyle (pb/m); polar granule (pg); Stieda (sb) and vestigial sub-Stieda (ssb) bodies; sporocyst residuum (sr); anterior (arb) e posterior (prb) refractile bodies. All to same scale. Scale-bar: 10 µm

Representative DNA sequence: One representative COI sequence was deposited in the GenBank database under the accession number OM933654.

Remarks: The morphology of *Eimeria* spp. recorded from the order Pelecaniformes, in addition to the orders Suliformes and Phaethontiformes, which were related to Pelecaniformes, and of *E. bazi* identified from *T. caudatus* in this study are shown in Table 1. Nine *Eimeria* spp. are recorded in these orders: *Eimeria ardeae* Dubinin, 1939, *E. bazi*, *Eimeria pelecani* Courtney et Ernst, 1975, *Eimeria ardae* Shamsuddin et Jasim, 1980 and *Eimeria garzettae* Golemansky et Kuldjieva, 1980 from Pelecaniformes and *Eimeria roscoviensis* (Labbé, 1893), *Eimeria urnula* Hoare, 1933, *Eimeria auritusi* Yabsley, Gottdenker et Fisher, 2002 and *Eimeria phalacrocoraxae* Yabsley et Gibbs, 2006 from Suliformes. From Phaethontiformes there are no described species. As shown in Table 1, the oocysts recovered from *T. caudatus* in this study were morphologically similar with the original description of *E. bazi* from *P. papillosa* in India. In addition, only *E. roscoviensis*, *E. urnula*, *E. garzettae* and *E. phalacrocoraxae* have a micropyle and oocyst shape that are reasonably similar to the oocysts of this study; however, these species can be distinguished by their smaller sizes, pear-shaped oocysts, non-protruding micropyle and absence of polar granules in the sporulated oocyst (Table 1).

Phylogenetic analysis: DNA amplification of the oocyst of *E. bazi* showed a clear band of *c.*250 bp. Phylogenetic analysis included 20 sequences for coccidians available on GenBank (Fig. 3). *Hepatozoon canis* (James, 1905) was used as the outgroup. *Eimeria bazi* sat separately on the cladogram for having low similarity with coccidians sequenced for the same genic region deposited in GenBank. The highest similarity was only 88.4% with *Isospora oliveirai* Ortúzar-Ferreira et Berto, 2020, which is a coccidian parasite of Neotropical passerines [16]. The closest eimerian sequence was of *Eimeria bubonis* Cawthorn et Stockdale, 1981, an owl parasite, with 86.3% similarity [17]; followed by *Eimeria cahirinensis* Couch, Blaustein, Duszynski, Shenbrot et Nevo, 1997, *Eimeria columbinae* Ortúzar-Ferreira et Berto, 2020, *Eimeria purpureicephali* Yang, Brice et Ryan, 2016 and *Eimeria dispersa* Tyzzer, 1929, which are parasites of rodents, columbiformes, psittaciformes and galliformes, respectively, with 85–86% similarity [18–21].

Discussion

The study of parasitology presupposes, among other things, the biological understanding of both the parasite and its host, because the dynamics of this ecological interaction between parasite–host can occur in a variety of ways [22, 23]. There

Table 1 Comparative morphology of *Eimeria* spp. recorded from the orders Pelecaniformes and Suliformes

Coccidia	Hosts	References	Oocysts								Sporocysts						
			Shape	Length (µm)	Width (µm)	L/W ratio	Wall (µm)	Micropyle (µm)	Polar granule	Residuum	Shape	Length (µm)	Width (µm)	L/W ratio	Stieda body (µm)	Sub-stieda body	Residuum (µm)
Eimeria roscoviensis (Labbé, 1893)	*Gulosus aristotelis* (Linnaeus, 1761) (Suliformes: Phalacrocoracidae)	Labbé [40]	Pear-shaped	16–18	14–16	–	Smooth	Inconspicuous or incomplete, with polar body	Absent	Absent	Pear-shaped	(9)	(6)	–	Present, knob-like	Absent	Membrane-bound
Eimeria urnula Hoare, 1933	*Phalacrocorax carbo* (Linnaeus, 1758) (Suliformes: Phalacrocoracidae)	Hoare [41]	Roughly ovoidal or pear-shaped	18–23	13–14	–	Smooth	Inconspicuous or incomplete	Absent	Absent	Ovoidal or ellipsoidal	(10.4)	(5.6)	–	Present	Absent	Membrane-bound, rounded
Eimeria ardeae Dubinin, 1939	*Ardea cinerea* Linnaeus, 1758 and *Nycticorax nycticorax* (Linnaeus, 1758) (Pelecaniformes: Ardeidae)	Dubinin [45]	Ovoidal	45–57	32–48	–	–	Indiscernible	–	–	Elongate ovoidal	24–28	–	(0.5)	Present	–	Large
Eimeria bazi Chauhan et Bhatia, 1970	*Pseudibis papillosa* (Temminck, 1824) (Pelecaniformes: Threskiornithidae)	Chauhan and Bhatia [10]	Ovoidal or ellipsoidal	21–28 (23.7)	16–20 (16.8)	1.2–1.7 (1.4)	Smooth, (0.7)	Proeminent, 3.9–4.5	Present, 1	Absent	Ellipsoidal	10.4–13.0 (12.2)	6.5–7.8 (7.2)	–	Present, small knob-like	absent	Mass of dark granules
	Theristicus caudatus (Boddaert, 1783) (Pelecaniformes: Threskiornithidae)	Current study	Ovoidal	21–30 (26.2)	16–21 (18.9)	1.2–1.6 (1.39)	Smooth to slightly rough, 1.5–1.9 (1.7)	Robust and protruding, 4.1–5.4 (4.8), occasionally with polar body	Present, small, 1 or 2	Absent	Ovoidal to lemon-shaped	13–15 (14.2)	7–10 (8.7)	1.4–1.8 (1.65)	Present, knob-like to rounded, 0.7–1.4 × 1.3–1.9 (1.1 × 1.5)	Absent or vestigial	Membrane-bound, 3.4–5.5 × 3.5–4.3 (4.7 × 4.0)

Table 1 (continued)

Coccidia	Hosts	References	Oocysts								Sporocysts						
			Shape	Length (μm)	Width (μm)	L/W ratio	Wall (μm)	Micropyle (μm)	Polar granule	Residuum	Shape	Length (μm)	Width (μm)	L/W ratio	Stieda body (μm)	Sub-stieda body	Residuum (μm)
Eimeria pelecani Courtney et Ernst, 1975	*Pelecanus occidentalis* Linnaeus, 1766 (Pelecaniformes: Pelecanidae)	Courtney and Ernst [46]	Broadly pear-shaped to ovoidal	14–20 (17.6)	13–16 (14.2)	1.0–1.5 (1.24)	pitted, (1.1)	Absent	Present, 1	Absent	Pear-shaped	10–13 (11.0)	5–8 (6.7)	–	Present, small	Absent	Membrane-bound, spherical, 4–5 × 4–5
Eimeria ardae Shamsuddin et Jasim, 1980	*Ardea purpurea* Linnaeus, 1766 (Pelecaniformes: Ardeidae)	Shamsuddin and Jasim [47]	Subspherical	14.9–19.8 (17.6)	12.4–18.2 (15.0)	1.0–1.4 (1.2)	Pitted, (1.4)	Indistinct	Present, 1	Absent	Ovoidal	6.6–9.9 (8.0)	5.8–8.6 (6.4)	1.1–1.5 (1.3)	Present	Absent	Central, composed of fine granules
Eimeria garzettae Golemansky et Kuldjieva, 1980	*Egretta garzetta* (Linnaeus, 1766) (Pelecaniformes: Ardeidae)	Golemansky and Kuldjieva [44]	Subspherical or ovoidal	17.6–21.2	14.4–16.2	–	Smooth to sculptured, 1.2–2.0	Distinct, with polar body of 3 μm	Present, 1–2	Absent	Pear-shaped	9–11	6–8	–	Present, pointed	Absent	Compact
Eimeria auritusi Yabsley, Gottdenker et Fisher, 2002	*Nannopterum auritus* (Lesson, 1831) (Suliformes: Phalacrocoracidae)	Yabsley *et al.* [48]	Subspherical	14–19 (16.5)	14–18 (16.1)	1.0–1.2 (1.0)	Pitted, (1.0)	Absent	Absent	Present, small, 4–8 granules	Ovoidal	8–11 (9.3)	6–7 (6.6)	(1.4)	Absent	Absent	Scattered
Eimeria phalacrocoraxae Yabsley et Gibbs, 2006	*Phalacrocorax auritus* (Lesson, 1831) (Suliformes: Phalacrocoracidae)	Yabsley and Gibbs [42]	Ovoidal	16–18.5 (17.1)	13–17 (14.7)	1.03–1.29 (1.17)	Smooth, with bumps, (1.0)	Prominent, 4.0–4.5, with large polar body	Absent	Absent	Ovoidal	8.5–10.5 (9.6)	5.0–6.5 (5.9)	1.3–1.8 (1.63)	Present, small	Absent	Membrane-bound

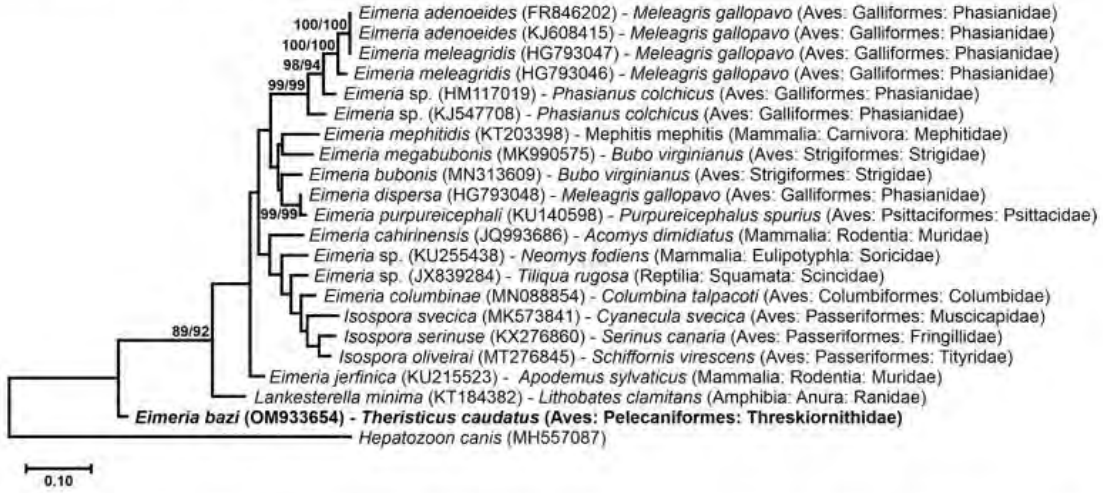

Fig. 3 Maximum likelihood tree estimated from the COI sequences. Numbers at nodes represent bootstrap support (1000 replicates; only values > 50% shown) for neighbor-joining and maximum likelihood, respectively. The scale-bar represents the number of nucleotide substitutions per site

are parasites that are specific to a group of susceptible hosts, while others parasitize a wide range of hosts, thus being generalists [22]. The overwhelming part of the scientific literature on eimeriid coccidia follows the guidelines of Duszynski and Wilber [11], that these parasites are minimally specific at the familial level of their hosts; therefore, to describe a new species, it is necessary to have a detailed morphological comparison with other congeneric species already described for the same host family [11].

So far only *E. bazi* has been recorded in the Threskiornithidae family. This coccidian species was originally described in Asia, from red-naped ibises *P. papillosa*, which have a geographic distribution restricted to India, Nepal and Pakistan [24]. In this context, it is unlikely that *E. bazi* was directly transmitted from *P. papillosa* to *T. caudatus*, as these birds are allopatric inhabiting distinct and distant continents. On the other hand, many ibises are migrants and/or have very wide geographic distributions, being observed on all continents, with the exception of Antarctica [25]. The glossy ibis *Plegadis falcinellus* (Linnaeus, 1766), which is observed in Oceania, Asia, Africa, Europe and in South, Central and North America, can be highlighted by its migratory potential and wide intercontinental distribution [24]. The Eurasian spoonbill *Platalea leucorodia* Linnaeus, 1758, in turn, is distributed as resident in Europe, Asia and Africa, but is reported as vagrant in Brazil [24]. Thus, these and other migrant and/or intercontinental ibises could be transmissors/dispersers of coccidians for susceptible threskiornithid birds on all continents, including *P. papillosa* and *T. caudatus*, which have more restricted distributions [26]. Studies such as those by Silva *et al.* [27] and Silva-Carvalho *et al.* [28] exemplify the dispersal potential of coccidian parasites. In addition, the report of eimerian oocysts from northern bald ibises *Geronticus eremita* (Linnaeus, 1758) and African sacred ibises *Threskiornis aethiopicus* (Latham, 1790), which records at least one *Eimeria* sp. on the African continent from Threskiornithidae reinforces the assumption of the intercontinental dispersion of *E. bazi*; however, it would be unwise to identify these eimerian oocysts of these african ibises as *E. bazi* outside India, as in these studies none of the oocysts were specifically identified, described, or named [29–31].

Another possibility that should be considered is the anthropogenic dispersion of coccidia through the trade/trafficking of wild animals [26]. An example is the zoos and breeding sites that are qualified to receive wild animals from the most diverse parts of the world. *Isospora araponga* Doležalová, Torres, Fernández et Modrý, 2004 was described from bare-throated bellbirds *Procnias nudicollis* (Vieillot, 1817) imported to Barcelona Zoo from Brazil [32]. Berto *et al.* [33] reported *Tyzzeria parvula* (Kotlán, 1933) in Brazil from greylag geese *Anser anser* (Linnaeus, 1758), an Asian species that is commonly traded as a domestic animal in several regions of the world [24]. In this way, the trade/trafficking of wild animals can allow the dispersion/transmission of coccidia among naturally allopatric birds.

Still on the geografic distribution of ibises, it is worth mentioning that *T. caudatus*, despite being typical of open environments, such as cerrado and fields, has expanded its geographic distribution in recent years to other biomes, which were originally forested, such as the Atlantic Forest [5, 7, 34]. This biogeographic expansion has been potentiated by changes in these habitats resulting from deforestation, in addition to the large increase in the creation of

pasture fields, providing the emergence of environments similar to the original habitat of this bird [35]. Thus, these new open areas of anthropogenic origin provide an ideal habitat for the permanence of *T. caudatus*. One of the consequences of this expansion of the geographic distribution of *T. caudatus* is the dispersion of its coccidia in new areas, including for new host species that previously would not have sympatry with this bird in its native habitat. Along with that, the very high densities of oocysts (above 100,000), observed in some droplets of feces collected from *T. caudatus* in the present study may cause epizootic diseases in host populations, especially those without previous contact with the parasite [26]. This may occur considering that the symptoms of coccidiosis must be closely related to the amount of oocysts ingested, in addition to other factors [2, 36].

In the absence or when there is a low number of descriptions of coccidian species in a host family is suitable the comparison with coccidian species recorded in higher taxa of the host [16]. Thus, although the oocysts recovered from *T. caudatus* in the current study are morphologically identifiable as *E. bazi*, which is the only recorded species of the host family Threskiornithidae, these were also compared with the coccidians described in the next higher taxon, in this case, the order Pelecaniformes. Recent studies have shown that the traditional Pelecaniformes are actually a polyphyletic group; therefore, several taxonomic rearrangements were proposed in a new classification that included two new orders, Suliformes and Phaethontiformes, from the traditional Pelecaniformes. Furthermore, it is noteworthy that the family Threskiornithidae belonged to the order Ciconiiformes; however, currently the order Ciconiiformes has only the family Ciconiidae, and Threskiornithidae along with other families were reclassified to the order Pelecaniformes [4, 6, 37–39]. Thus, aiming to expand the comparative morphology of *E. bazi*, *Eimeria* spp. recorded from Pelecaniformes, Suliformes and Phaethontiformes were included in this study, obtaining nine *Eimeria* spp. for morphological comparison (Table 1).

The first described species of these traditional Pelecaniformes was *Eimeria roscoviensis* (Labbé, 1893) from European shags *Gulosus aristotelis* (Linnaeus, 1761) in Roscoff, France. This species was originally described as *Coccidium roscoviense* Labbé, 1893 before being correctly classified in the genus *Eimeria* [40]. Two later described species of birds of the same host family (Phalacrocoracidae), *Eimeria urnula* Hoare, 1933 and *Eimeria phalacrocoraxae* Yabsley et Gibbs, 2006, are very similar to each other, both morphometrically and morphologically, mainly due to the typical micropyle with a polar body (Table 1) [41, 42]. This observation shows the possibility of these coccidians being a single species, or being closely related species that have co-evolved from an ancestral eimeriid that parasitized an ancestral bird of the Phalacrocoracidae, in the process named co-speciation [26, 43]. In the oocysts identifiable as *E. bazi* in this study, as well as in its original description [10] and in *E. garzettae* [44], this same type of oocyst shape and size, including the micropyle with polar body, are observed (Table 1), showing that perhaps the common ancestor of these *Eimeria* spp. may have started parasitism even earlier in the evolution of Pelecaniformes and Suliformes [26, 43]. This assumption is reinforced by the findings of Labbé from the description of *E. roscoviensis*, still in the late nineteenth century, who observed oocysts of the same morphotype as *E. roscoviensis* from Charadriiformes of the genera *Charadrius* Linnaeus, 1758, *Arenaria* Brisson, 1760, *Numenius* Brisson, 1760, *Tringa* Linnaeus, 1758 and *Calidris* Merrem, 1804, showing that the diversity of hosts susceptible to these *Eimeria* spp. with polar bodies attached to the micropyle may be even larger [40].

The other species *Eimeria ardeae* Dubinin, 1939, *Eimeria pelecani* Courtney et Ernst, 1975, *Eimeria ardae* Shamsuddin et Jasim, 1980 and *Eimeria auritusi* Yabsley, Gottdenker et Fisher, 2002 do not have the typical morphology observed in the previous species, as they do not have micropyle and are subspherical as in the case of *E. auritusi* and *E. ardae*; therefore, these must have another evolutionary origin [45–48]. The answers to these phylogenetic questions of these *Eimeria* spp. of Pelecaniformes, Suliformes, and also Charadriiformes, could be conclusive by means of phylogenetic molecular analyses. However, of all *Eimeria* spp. related in this study, only *E. auritusi* and *E. phalacrocoraxae* were sequenced for a region of the 18S small subunit ribosomal RNA (18S) gene, unlike in this study, where *E. bazi* was sequenced for a genic region of the COI, and therefore, these species did not appear in the cladogram of Fig. 3. The sequencing of the 18S gene was intended in this study, but its amplification was not successful, perhaps due to the use of DNA extraction methodology from an individual oocyst that provides few copies of nuclear DNA, unlike the greater amount of mitochondrial DNA available in each oocyst. In any case, the use of the 18S gene for species differentiation and detection of recent evolutionary events has been shown to be unsuitable, whereas the COI gene has been considered the most suitable in this sense [49, 50]. Thus, when a greater number of *Eimeria* spp. of these birds have been sequenced, the phylogenetic analysis should be more conclusive, as, so far, the phylogenetic analysis for the COI gene has only shown that *E. bazi* is distant and paraphyletic from some coccidia groups of passeriform and non-passeriform birds (Fig. 3).

Finally, after the detailed morphological study of the oocysts recovered from *T. caudatus* in the current study and considering the migratory potential and worldwide distribution of ibises, in addition to other possibilities, *E. bazi* is reported for the first time in South America from a new host, *T. caudatus*. As an additional element, this study provided

the first genetic sequencing by the COI gene for this coccidian species.

Acknowledgements We thank Dr. Donald W. Duszynski (Emeritus Professor of Biology, University of New Mexico), the Biblioteca de Ciências Biomédicas of the Fundação Oswaldo Cruz, the Central Library of the Universidade Federal Rural do Rio de Janeiro, and the Library of the Museu Nacional of the Universidade Federal do Rio de Janeiro, for providing us with old and rare scientific articles that were essential for the conclusion of this study.

Author contributions The study was designed by VML, IF and BPB. Field work was performed by IF and RBGC. Laboratory procedures for maintenance, recovery, measurements, photomicrographs and isolation of oocysts were performed by RBGC, CNO-F and MSO. DNA extraction, amplification and sequencing were performed by ERM, AAO and VML. BPB analyzed the data and drew the coccidian oocyst. The manuscript was written by RBGC, CNO-F and BPB and subsequently revised by all other authors. All authors read and approved the final manuscript.

Funding This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ). RBGC has a scholarship from CNPq (Grant/Award number: 170041/2018-2). CNO-F has a scholarship from CNPq/UFRRJ (Grant/Award Number: PVBS2485-2021). MSO has a post-doctoral scholarship from FAPERJ (Grant/Award number: E-26/204.228/2021). BPB has fellowships from CNPq (Grant/Award number: 303899/2019-0) and from FAPERJ (Grant/Award number: E-26/202.797/2019).

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Field-collecting permits were issued by SISBIO/ICMBio (license 42798) and CEUA/IV/UFRRJ (protocol 6606250616). All applicable institutional, national and international guidelines for the care and use of animals were followed.

References

1. Ruggiero MA, Gordon DP, Orrell TM, Bailly N, Bourgoin T, Brusca RC, Cavalier-Smith T, Guiry MD, Kirk PM (2015) Correction: a higher level classification of all living organisms. PLoS ONE 10:e0130114. https://doi.org/10.1371/journal.pone.0119248
2. Fayer R (1980) Epidemiology of protozoan infection the coccidia. Vet Parasitol 6:75–103. https://doi.org/10.1016/0304-4017(80)90039-4
3. Duszynski DW, Couch L, Upton SJ (2000) The Coccidia of the World. https://www.k-state.edu/parasitology/worldcoccidia. Accessed 1 Mar 2022
4. Clements JF, Schulenberg TS, Iliff MJ, Billerman SM, Fredericks TA, Gerbracht JA, Lepage D, Sullivan BL, Wood CL (2021) The eBird/clements checklist of birds of the World: version 2021. https://www.birds.cornell.edu/clementschecklist/download/. Accessed 01 March 2022.
5. Matheu E, delHoyo J, Garcia EFJ, Boesman PFD (2020) Buff-necked Ibis (*Theristicus caudatus*), version 1.0. In: delHoyo J, Elliott A, Sargatal J, Christie DA, de Juana E (eds) Birds of the World. Cornell Lab of Ornithology, Ithaca. https://doi.org/10.2173/bow.bunibi1.01. Accessed 1 Mar 2022.
6. Sick H (1997) Ornitologia Brasileira. Nova Fronteira, Rio de Janeiro
7. Serpa GA, Pacheco JF, Lima LM, Parrini R, Pimentel LS, Pinto MFR, Antonini RD, Rajão H, Oliveira AH, Tavares DC, Siciliano S, Mallet-Rodrigues F, Luz HR, Soares BR, Crud N (2010) A curicaca, *Theristicus caudatus* (Ciconiiformes: Threskiornithidae) no estado do Rio de Janeiro: revisão dos registros e novas observações. Atual Ornitol 153:62–68
8. Cabral RBG, Ferreira I (2012) Primeiro registro de *Theristicus caudatus* (Pelecaniformes, Threskiornithidae) em Seropédica. RJ Atual Ornitol 165:10–11
9. Dolnik OV, Dolnik VR, Bairlein F (2010) The effect of host foraging ecology on the prevalence and intensity of coccidian infection in wild passerine birds. Ardea 98:97–103. https://doi.org/10.5253/078.098.0112
10. Chauhan PPS, Bhatia BB (1970) Eimerian oocysts from *Pseudibis papillosa*. Indian J Microbiol 10:53–54
11. Duszynski D, Wilber P (1997) A guideline for the preparation of species descriptions in the Eimeriidae. J Parasitol 83:333–336. https://doi.org/10.2307/3284470
12. Berto BP, McIntosh D, Lopes CWG (2014) Studies on coccidian oocysts (Apicomplexa: Eucoccidiorida). Rev Bras Parasitol Vet 23:1–15. https://doi.org/10.1590/S1984-29612014001
13. Dolnik OV, Palinauskas V, Bensch S (2009) Individual oocysts of *Isospora* (Apicomplexa: Coccidia) parasites from avian faeces: from photo to sequence. J Parasitol 95:169–174. https://doi.org/10.1645/GE-1873.1
14. Yang R, Brice B, Elliot A, Ryan U (2015) *Isospora serinuse* n. sp. (Apicomplexa: Eimeriidae) from a domestic canary (*Serinus canaria* forma domestica) (Passeriformes: Fringillidae) in Western Australia. Exp Parasitol 159:59–66. https://doi.org/10.1016/j.exppara.2015.08.020
15. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096
16. Ortúzar-Ferreira CN, Genovez-Oliveira JL, Oliveira MS, Mello ER, Thode-Filho S, Oliveira ÁA, Lima VM, Ferreira I, Berto BP (2020) *Isospora oliveirai* n. sp. (Chromista: Miozoa: Eimeriidae) from the greenish schiffornis *Schiffornis virescens* (Lafresnaye, 1838) (Passeriformes: Tyranni: Tityridae) in South America. Acta Parasitol 65:843–851. https://doi.org/10.1007/s11686-020-00237-8
17. McAllister CT, Hnida JA, Woodyard ET, Rosser TG (2019) *Eimeria* spp. (Apicomplexa: Eimeriidae) from great horned owls, *Bubo virginianus* (Gmelin) (Aves: Strigiformes) from Arkansas and Oklahoma, USA, with novel molecular information on *Eimeria bubonis* Cawthorn & Stockdale, 1981. Syst Parasitol 96:695–702. https://doi.org/10.1007/s11230-019-09881-9
18. Kvičerová J, Hypša V (2013) Host-parasite incongruences in rodent *Eimeria* suggest significant role of adaptation rather than cophylogeny in maintenance of host specificity. PLoS ONE 8:e63601. https://doi.org/10.1371/journal.pone.0063601
19. Vrba V, Pakandl M (2014) Coccidia of turkey: from isolation, characterisation and comparison to molecular phylogeny and molecular diagnostics. Int J Parasitol 44:985–1000. https://doi.org/10.1016/j.ijpara.2014.06.004
20. Yang R, Brice B, Ryan U (2016) Morphological and molecular characterization of *Eimeria purpureicephali* n. sp. (Apicomplexa: Eimeriidae) in a red-capped parrot (*Purpureicephalus spurius*, Kuhl, 1820) in Western Australia. Int J Parasitol Parasites Wildl 5:34–39. https://doi.org/10.1016/j.ijppaw.2016.01.003

21. Ortúzar-Ferreira CN, Oliveira MS, Genovez-Oliveira JL, Franco HA, Thode-Filho S, Cardozo SV, Oliveira ÁA, Lima VM, Ferreira I, Berto BP (2020) Coccidia of Columbiformes: a taxonomic review of its Eimeriidae species and *Eimeria columbinae* n. sp. from *Columbina talpacoti* (Temminck, 1809) from Brazil. Parasitol Res 119:267–281. https://doi.org/10.1007/s00436-019-06514-4
22. Ferreira LF (1973) O fenômeno parasitismo. Rev Soc Bras Med Trop 3:261–277. https://doi.org/10.1590/S0037-86821973000400006
23. Atkinson CT, Thomas NJ, Hunter DB (2008) Parasitic diseases of wild birds. Wiley-Blackwell, Singapore
24. BirdLife International (2016) The IUCN red list of threatened species. http://www.iucnredlist.org. Accessed 23 May 2022
25. Billerman M, Keeney BK, Rodewald PG, Schulenberg TS (2022) Birds of the World. Cornell Lab of Ornithology, Ithaca. https://birdsoftheworld.org/bow/home. Accessed 23 May 2022
26. Berto BP, Lopes CWG (2020) Coccidia of wild birds as ecological biomarkers: some approaches on parasite-host-environment interaction. J Parasitol 106:707–713. https://doi.org/10.1645/19-148
27. Silva LM, Rodrigues MB, Pinho IF, Lopes BB, Luz HR, Ferreira I, Lopes CWG, Berto BP (2017) Some remarks on the distribution and dispersion of coccidia from icterid birds in South America: *Isospora guaxi* n. sp. and *Isospora bellicosa* Upton, Stamper & Whitaker, 1995 (Apicomplexa: Eimeriidae) from the red-rumped cacique *Cacicus haemorrhous* (L.)(Passeriformes: Icteridae) in Southeastern Brazil. Syst Parasitol 94:151–157. https://doi.org/10.1007/s11230-016-9688-y
28. Silva-Carvalho LM, Pastura DG, Rodrigues MB, Gomes JV, Oliveira MS, Siqueira PB, Genovez-Oliveira JL, Soares SS, Oliveira ÁA, Lima VM, Ferreira I, Berto BP (2018) *Isospora sagittulae* McQuistion & Capparella, 1992 (Apicomplexa: Eimeriidae) from antbirds (Passeriformes: Thamnophilidae) in the Amazon and Atlantic Forest of Brazil: with notes on its distribution and dispersion in the Neotropical region. Parasitol Res 117:2635–2641. https://doi.org/10.1007/s00436-018-5955-y
29. Frigerio D, Cibulski L, Ludwig SC, Campderrich I, Kotrschal K, Wascher CAF (2016) Excretion patterns of coccidian oocysts and nematode eggs during the reproductive season in Northern Bald Ibis (*Geronticus eremita*). J Ornithol 157:839–851. https://doi.org/10.1007/s10336-015-1317-z
30. Parsani HR, Momin RR, Sahu RK, Patel BG (2003) Prevalence of gastro-intestinal parasites in captive birds at Kalma Nehru Zoological Garden, Kankaria Zoo, Ahmedabad, Gujarat. Zoo's Print J 18:987–992
31. Bastian S, Yesou P, Clergeau P, Laroucau K, Pellerin JL, Hars J, Bazus J, Passet A, Lagrange P, L'Hostis M (2010) Eléments pour l'évaluation des risques sanitaires liés aux Ibis sacrés (*Threskiornis aethiopicus*) en France. Rapport d'étude pour la Direction Régionale de l'Environnement Bretagne et la Direction Régionale de l'Environnement, de l'Aménagement et du Logement des Pays de la Loire. https://controverses.minesparis.psl.eu/public/promo15/promo15_G6/www.oncfs.gouv.fr/IMG/pdf/rapport_ibis_pathogenes.pdf. Accessed 1 Mar 2022
32. Doležalová M, Torres J, Fernández H, Modrý D (2004) *Isospora araponga* sp. n. (Apicomplexa: Eimeriidae), a new species of *Isospora* Schneider from a bare-throated bellbird, *Procnias nudicollis* (Vieillot, 1817) (Passeriformes: Cotingidae) from Brazil. Mem Inst Oswaldo Cruz 99:829–830. https://doi.org/10.1590/S0074-02762004000800008
33. Berto BP, Teixeira M, Lopes CWG (2007) *Tyzzeria parvula* (Kotlán, 1933) Klimes, 1963 (Apicomplexa: Eimeriidae) in the greylag goose (*Anser anser* Linnaeus, 1758) in southeastern Brazil. Rev Bras Parasitol Vet 16:156–158. https://doi.org/10.1590/S1984-29612007000300008
34. WikiAves (2022) Wiki Aves—A Enciclopédia das Aves do Brasil. https://www.wikiaves.com.br/wiki/curicaca. Accessed 01 Mar 2022
35. Sigrist T (2014) Guia de Campo: Avifauna Brasileira. Avis Brasilis, São Paulo
36. Eckert J, Braun R, Shirley MW, Coudert P (1995) Biotechnology—guidelines on techniques in Coccidiosis Research. European Commission, Luxembourg
37. Ericson PGP, Anderson CL, Britton T, Elzanowski A, Johansson US, Källersjö M, Ohlson JL, Parsons TJ, Zuccon D, Mayr G (2006) Diversification of Neoaves: integration of molecular sequence data and fossils. Biol Lett 2:543–547. https://doi.org/10.1098/rsbl.2006.0523
38. Hackett SJ, Kimball RT, Reddy S, Bowie RCK, Braun EL, Braun MJ, Chojnowski JL, Cox WA, Han KL, Harshman J, Huddleston CJ, Marks BD, Miglia KJ, Moore WS, Sheldon FH, Steadman DW, Witt CC, Yuri T (2008) A phylogenomic study of birds reveals their evolutionary history. Science 320:1763–1768. https://doi.org/10.1126/science.1157704
39. Mayr G (2011) Metaves, Mirandornithes, Strisores and other novelties—a critical review of the higher-level phylogeny of neornithine birds. J Zool Syst Evol Res 49:58–76. https://doi.org/10.1111/j.1439-0469.2010.00586.x
40. Labbé A (1893) Sur les coccidies des oiseaux. C R Seances Acad Sci 117:407–409
41. Hoare CA (1933) Studies on some new ophidian and avian coccidian from Uganda, with a revision of the classification of the Eimeriidae. Parasitol 24:359–388. https://doi.org/10.1017/S0031182000019569
42. Yabsley MJ, Gibbs SE (2006) Description and phylogeny of a new species of *Eimeria* from double-crested cormorants (*Phalacrocorax auritus*) near Fort Gaines, Georgia. J Parasitol 92:385–388. https://doi.org/10.1645/GE-592R.1
43. Gardner SL, Duszynski DW (1990) Polymorphism of eimerian oocysts can be a problem in naturally infected hosts: an example from subterranean rodents in Bolivia. J Parasitol 76:805–811 (**3282798**)
44. Golemansky V, Kuldjieva D (1980) *Eimeria garzettae* sp. n. (Coccidia: Eimeriidae) in the little egret (*Egretta garzetta*) from Bulgaria. Acta Protozoologica 19:177–179
45. Dubinin VB (1939) The coccidiosis of the herons in the state Astrakhan reservate. Seriya Biologischeskikh Nauk 11:44–57
46. Courtney CH, Ernst JV (1975) *Eimeria pelecani* sp. n. from the brown pelican, *Pelecanus occidentalis carolinensis*, from Florida. J Parasitol 61:1081–1082. https://doi.org/10.2307/3279380
47. Shamsuddin M, Jasim MK (1980) Coccidia of some birds and mammals from Iraq. Bull Nat Hist Res Centre 7:81–109
48. Yabsley MJ, Gottdenker NL, Fischer JR (2002) Description of a new *Eimeria* sp. and associated lesions in the kidneys of double-crested cormorants (*Phalocrocorax auritus*). J Parasitol 88:1230–1233. https://doi.org/10.1645/0022-3395(2002)088[1230:DOANES]2.0.CO;2
49. Ogedengbe JD, Hanner RH, Barta JR (2011) DNA barcoding identifies *Eimeria* species and contributes to the phylogenetics of coccidian parasites (Eimeriorina, Apicomplexa, Alveolata). Int J Parasitol 41:843–850. https://doi.org/10.1016/j.ijpara.2011.03.007
50. Genovez-Oliveira JL, Oliveira MS, Thode-Filho S, Cardozo SV, Oliveira ÁA, Lima VM, Ferreira I, Berto BP (2020) Morphological and molecular identification of *Isospora massardi* Lopes, Berto, Luz, Galvão, Ferreira & Lopes, 2014 (Chromista: Miozoa: Eimeriidae) from thrushes *Turdus* spp. (Passeriformes: Turdidae) in South America. Parasitol Int 75:102040. https://doi.org/10.1016/j.parint.2019.102040

Authors and Affiliations

Rísia Brígida Gonçalves Cabral[1] · Carlos Nei Ortúzar-Ferreira[2] · Mariana de Souza Oliveira[1] · Ericson Ramos de Mello[3] · Águida Aparecida de Oliveira[4] · Viviane Moreira de Lima[5] · Ildemar Ferreira[5] · Bruno Pereira Berto[5]

1 Programa de Pós-graduação em Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil

2 Curso de Graduação em Medicina Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil

3 Programa de Pós-graduação em Ciências Veterinárias, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil

4 Departamento de Microbiologia e Imunologia Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil

5 Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, BR 465, Km 7, Seropédica, Rio de Janeiro 23890-000, Brazil

Zootaxa 5168 (1): 083–091
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5168.1.7
http://zoobank.org/urn:lsid:zoobank.org:pub:898E505A-453D-4935-8341-72F10F0B7475

Molecular identification of *Isospora coerebae* Berto, Flausino, Luz, Ferreira & Lopes, 2010 (Chromista: Miozoa: Eimeriidae) from the bananaquit *Coereba flaveola* (Linnaeus, 1758) (Passeriformes: Thraupidae: Coerebinae) from Brazil

CARLOS NEI ORTÚZAR-FERREIRA[1], LUCAS DE ASSIS S. ANDRADE[2], JHON LENNON GENOVEZ-OLIVEIRA[2], MARIANA S. OLIVEIRA[2], ERICSON R. MELLO[6], SERGIAN V. CARDOZO[7], ÁGUIDA A. OLIVEIRA[8], VIVIANE M. LIMA[9,10], ILDEMAR FERREIRA[9,11] & BRUNO P. BERTO[9,*]

[1]*Curso de Graduação em Medicina Veterinária, Universidade Federal Rural do Rio de Janeiro (UFRRJ), BR-465 km 7, 23897-000 Seropédica, RJ, Brazil.* ✉ *carlosneiortuzarferreira@gmail.com;* https://orcid.org/0000-0002-0177-5591
[2]*Programa de Pós-Graduação em Biologia Animal, UFRRJ, BR-465 km 7, 23897-000 Seropédica, RJ, Brazil*
[3]✉ *lucasandrade.as@hotmail.com;* https://orcid.org/0000-0001-8203-9299
[4]✉ *jhongenovezoliveira30@gmail.com;* http://orcid.org/0000-0003-2994-397X
[5]✉ *mariana.s.o@hotmail.com;* https://orcid.org/0000-0001-9594-0633
[6]*Programa de Pós-Graduação em Ciências Veterinárias, UFRRJ, BR-465 km 7, 23897-000 Seropédica, RJ, Brazil.*
✉ *melloericson@gmail.com;* https://orcid.org/0000-0002-7350-0562
[7]*Programa de Pós-Graduação em Biomedicina Translacional, Universidade do Grande Rio, Rua Professor José de Souza Herdy 1160, 25071-202 Duque de Caxias, RJ, Brazil.* ✉ *sergianvc@gmail.com;* https://orcid.org/0000-0003-2990-7936
[8]*Departamento de Microbiologia e Imunologia Veterinária, Instituto de Veterinária, UFRRJ, BR-465 km 7, 23897-000 Seropédica, RJ, Brazil.* ✉ *aguidaoliveira@gmail.com;* https://orcid.org/0000-0001-8685-0345
[9]*Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde, UFRRJ, BR-465 km 7, 23897-000 Seropédica, RJ, Brazil.*
[10]✉ *vilima@ufrrj.br;* https://orcid.org/0000-0001-7930-8312
[11]✉ *guaratibanofeliz@gmail.com;* https://orcid.org/0000-0002-6128-5789
[*]*Corresponding author.* ✉ *bertobp@ufrrj.br;* https://orcid.org/0000-0002-1072-5254

Abstract

Isospora coerebae Berto, Flausino, Luz, Ferreira & Lopes, 2010 is a coccidian protozoan described from bananaquits *Coereba flaveola* (Linnaeus, 1758), on Marambaia Island, which is located on the southeastern Brazilian coast. In this current work, *I. coerebae* is identified from *C. flaveola* in a protected area close to Marambaia Island, but on the mainland, establishing a new location of parasitism, in addition to providing a preliminary genotypic characterization via sequencing of two regions of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene. Its oocysts are sub-spherical, 24.4 × 22.9 µm, with smooth, bilayered wall, ~1.7 µm thick. Micropyle, polar granules and oocyst residuum are absent. Sporocysts are elongate ovoidal, 17.6 × 10.5 µm. Stieda body prominent and rounded and sub-Stieda body short and wide. Sporocyst residuum is composed of scattered granules of different sizes. Sporozoites are vermiform with a prominent posterior refractile body. The oocysts of the current work are morphologically equivalent to the original description of *I. coerebae*, which have a typical and easily identifiable morphology, mainly in the Stieda and Sub-Stieda bodies. The two sequenced gene regions of the COI gene approximated *I. coerebae* to *Isospora* spp. from Southeastern Brazil, but also from *Isospora* spp. from passerines of North America, Europe and Asia. Although there is a small overlap between the two genic regions sequenced in the current work, it is estimated that the longer COI sequence, which was recently designed and still not widespread, should show better phylogenetic results in the future.

Key words: taxonomy, genotypic characterization, sequencing, coccidia, *Isospora*, oocysts, Passeriformes, COI

Introduction

Coccidia are obligate intracellular protozoa, parasites of the intestinal tract of its hosts, with a cosmopolitan distribution and a biological cycle consisting of merogony, gametogony and sporogony (Levine 1985). These protozoans

Accepted by L. Pinto Utz: 29 Jun. 2022; published: 20 Jul. 2022

infect a wide range of hosts, from arthropods to mammals (Tenter *et al.* 2002; Ghemiri 2010). Coccidians of Eimeriidae are monoxenes, with *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881 (Berto *et al.* 2011; Berto & Lopes 2013) being the most representative genera in birds (Berto *et al.* 2011; Berto & Lopes 2013). In general, these microorganisms have an enzootic parasitic relationship, without major harm to its host or none at all. However, it is also known as the causative agents of coccidiosis, a parasitic disease that widely affects farm animals, causing economic losses and being strongly associated with environmental stress and poor management conditions (Fayer 1980; Júnior *et al.* 2009; Chapman *et al.* 2013).

The identification of coccidians is primarily conducted through the morphological identification of the oocysts, which are the exogenous structure of environmental resistance of the coccidians, shed in the feces of the host and infective when sporulated. However, currently the molecular studies have been increasingly required to provide molecular phylogenetic analysis and confirm and/or complement species identification (Tenter *et al.* 2002; Ogedengbe *et al.* 2011; Oliveira *et al.* 2021).

In this context, *Isospora coerebae* Berto, Flausino, Luz, Ferreira & Lopes, 2010 is a coccidian described by morphological and morphometric studies of their oocysts recovered from fecal samples of bananaquits *Coereba flaveola* (Linnaeus, 1758) in the Marambaia Island, on the Brazilian coast (Berto *et al.* 2010). Consequently, in this current work *I. coerebae* is identified from *C. flaveola* in a protected area close to Marambaia Island, but on the mainland, establishing a new parasitism locality, as well as providing a preliminary genotypic characterization via sequencing of two regions of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene.

Material and methods

Sample collection. A total of four expeditions were conducted in an environmental protection area, established in Brazil as a Private Natural Heritage Reserve (Reserva Particular do Patrimônio Natural - RPPN), named as Porangaba, in the Municipality of Itaguaí, in the State of Rio de Janeiro, Southeastern Brazil (22°48'29.83''S; 43°49'38.77''W). These were carried out in the months of July, August, September and October 2018. Only one bananaquit *C. flaveola* was captured with mist nets. The bananaquit was kept in individual boxes and feces collected immediately after defecation. After identification of the species (Sigrist 2014), the bird was photographed and released and fecal samples were placed in centrifuge tubes containing a potassium dichromate 2.5% ($K_2Cr_2O_7$) solution at 1:6 (v/v). Field-collecting permits were issued to B. P. Berto by SISBIO/ICMBio (license 42798) and CEUA/UFRRJ (protocol IV-6606250616).

Morphological analyses. Fecal samples were taken to the Laboratório de Biologia de Coccídios, Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro (UFRRJ), where they were incubated at room temperature (20–25°C) for one week. Oocysts were recovered by flotation in Sheather's sugar solution (Specific gravity: 1.20) and examined microscopically using the technique described by Duszynski & Wilber (1997) and Berto *et al.* (2014). Morphological observations, photomicrographs, and measurements were made with the use of an Olympus BX41 binocular microscope (Olympus Optical, Tokyo, Japan) coupled to a digital camera Eurekam 5.0 (BEL Photonics, Monza, Italy) connected to a computer running the software BELView (Version 6.2.3.0, BEL Engineering, Monza, Italy). All measurements are in micrometers and are given as the range followed by the mean in parentheses.

Isolation of an individual oocyst and DNA extraction. An individual oocyst was isolated from serial dilutions of the oocysts in drops on a microscope slide using a sterile micropipette. This isolated oocyst was resuspended in PBS and washed by centrifuging until the supernatant became clear (Dolnik *et al.* 2009). DNA was extracted from the oocyst using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, São Paulo, Brazil) according to the manufacturer's instructions. In order to fully lyse the oocysts, four freeze-thaw cycles were applied prior to the DNA extraction (Yang *et al.* 2014).

Amplification of ~250 bp of the COI gene (COI1). The PCR amplification of approximately 250 bp of the COI gene (COI1) was carried out using a nested PCR, as previously described by Dolnik *et al.* (2009) and Yang *et al.* (2015). The external primers: COIbF1 (5'-GWTCATTAGTATGGGCACATCA-3') and COIbR1 (5'-CCAAGAGATAATACRAARTGGAA-3'), produced a PCR product size of ~302pb. The internal primes: COIbF2 (5'-GGGCACATCATATGATGAC-3') and COIbR2 (5'-ATAGTATGTATCATGTARWGCAA-3') produced an amplicon of 257 bp in size. The PCR reaction contained 10µL of 5x Green GoTaq® Flexi Buffer, 3 µL of 25mM $MgCl_2$, 1 µL of

10mM dNTP´s, 0,4µM of each primer, 1.25 units of GoTaq® DNA polymerase, 3 µL of DNA (for primary reaction) or 3uL primary PCR product (for secondary reaction). Both primary and secondary PCR's were conducted using the same cycling conditions: 1 cycle of 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 47°C for 45 sec, and 72°C for 1min and a final extension of 72°C for 5min. The amplicons from the second round PCRs were purified using the Qiagen MinElute PCR Purification (Qiagen, São Paulo, Brazil).

Amplification of ~650 bp of the COI gene (COI2). The PCR amplification of approximately 650 bp of the COI gene (COI2) was carried out as previously described by Genovez-Oliveira *et al.* (2020). The primers: JAVF (5'-CTGAATTTGGTTCAGGTGTTGGT-3') and JAVR (5'-TACACCAGTAGTACCTCCAAGGG-3') produced a PCR product size of ~651pb. For amplification, a 25 µl PCR reaction was prepared using 3µL of genomic DNA (<1 µg), 12.5µL of GoTaq® G2 Hot Start Colorless Master Mix (Promega Labs) (1X), 0,25 µL of each Primer (0.2µM) and 9µL of Nuclease Free Water. PCR was conducted using the following cycling conditions: 1 cycle of 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 53°C for 45 sec, and 72°C for 50 sec and a final extension of 72°C for 5 min. The amplicons were purified using the Qiagen MinElute PCR Purification (Qiagen, São Paulo, Brazil).

DNA sequence analyses. All PCR products were sequenced using the PCR forward and reverse primers by Ludwig Biotechnology, where an ABI-Prism 3500 Genetic Analyzer (Applied Biosystems, Foster City, California) was used for Sanger sequencing. The results of the sequencing reactions were analyzed and edited using the program Chromas 2.6. Sequences were compared with other coccidian parasites available on the GenBank database using the Basic Local Alignment Search Tool (BLAST). Alignments were created in MEGA v10.2.6 using Clustal W (http://www.clustalw.genome.jp). Phylogenetic relationships were reconstructed using Bayesian Inference in the MrBayes v3.2.7 (Ronquist *et al.* 2012) and using Maximum likelihood method in the MEGA (Kumar *et al.* 2018). The best fitting evolutionary models for all phylogenetic analyses was selected by the Model Selection in MEGA. Bayesian Inference analysis was conducted under the GTR+G evolutionary model for 1,000,000 generations, and the trees were summarized after removing 25% of burn-in. Maximum likelihood analysis was conducted under the TN93+G evolutionary model, and the bootstrap values were calculated by 1,000 replicates. The resultant phylogenetic trees were visualized in the MrBayes and MEGA, exported in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/), and edited in Corel PHOTO-PAINT (Corel Draw Graphics Suite, Version 2020, Corel Corporation, Canada).

Results

The captured bananaquit *C. flaveola* was apparently healthy and eliminated oocysts of a single morphotype, which was morphologically identified as follows:

Isospora coerebae Berto, Flausino, Luz, Ferreira and Lopes, 2010

Host: Bananaquit *Coereba flaveola* (Linnaeus, 1758) (Passeriformes: Thraupidae: Coerebinae)

Locality: Private Natural Heritage Reserve of Porangaba (22°48'29.83''S; 43°49'38.77''W), Municipality of Itaguaí, State of Rio de Janeiro, Southeastern Brazil.

Specimens: Photomicrographs are deposited and available (http://r1.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under repository number 115/2021. Photovouchers of the host specimens are deposited in the same collection.

Representative DNA sequence: DNA amplification of the COI1 and COI2 genic regions showed clear bands around ~250 bp and ~650 bp, respectively. Representative sequences were deposited in the GenBank database under the accession numbers: OK194671 (COI1); and OK194672 (COI2).

Site of infection: Unknown.

Prevalence: 100% (1/1).

Sporulation: Exogenous. All oocysts were passed in the feces unsporulated and were fully sporulated by day 7 in $K_2Cr_2O_7$ solution at room temperature (20–25°C).

Morphology (Fig 1A-F): Oocyst (n = 15) sub-spherical, 22–27 × 22–25 (24.4 × 22.9); length/width (L/W) ratio 1.0–1.1 (1.07). Wall bi-layered, smooth outer wall about 2/3 total thickness, 1.5–1.8 (1.7). Micropyle, polar granules and oocyst residuum absent. Sporocyst elongate ovoidal, 16–19 × 10–11 (17.6 × 10.5); L/W ratio 1.6–1.8

(1.67). Stieda body present, prominent and rounded, 1.3−1.5 × 1.9−2.3 (1.4 × 2.1). Sub-Stieda body present, short and wide, 1.0−1.6 × 3.1−3.7 (1.3 × 3.5). Para-Stieda body absent. Crystalloid body sometimes present in the center of the sporocyst. Sporocyst residuum present, composed of many scattered granules of different sizes. Sporozoites vermiform, with a prominent posterior refractile body.

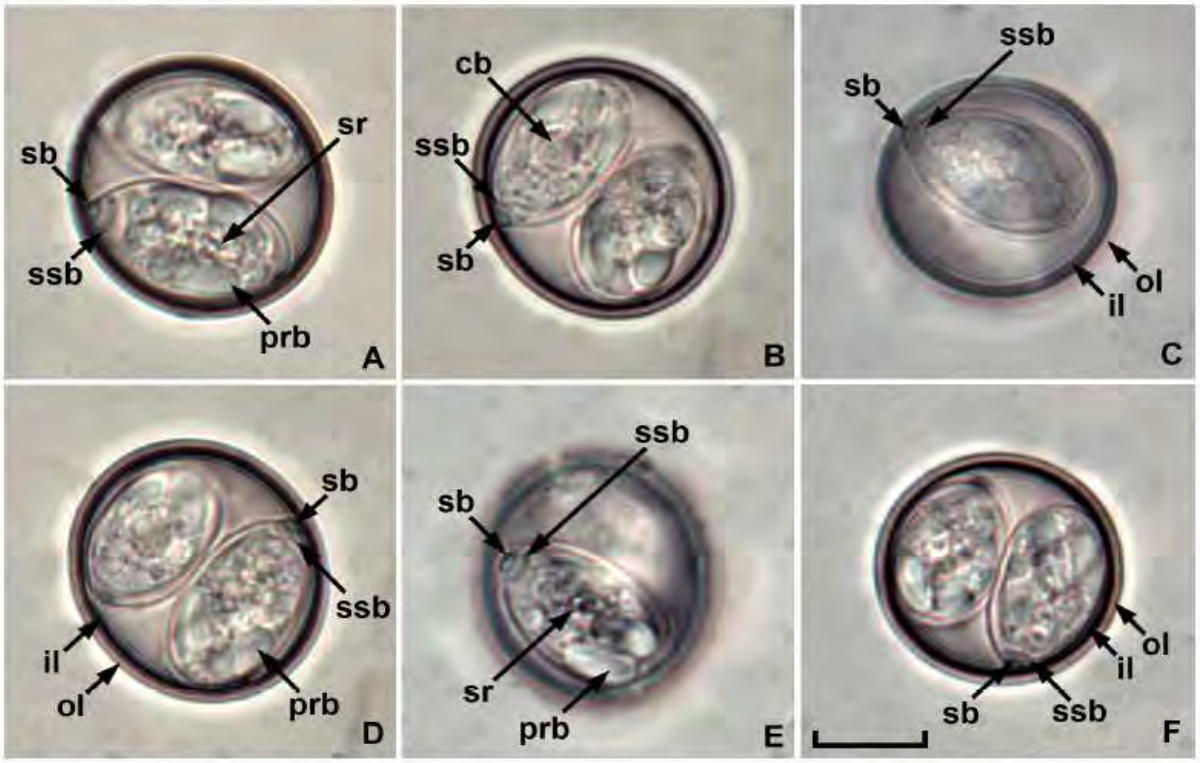

FIGURE 1. Photomicrographs of sporulated oocysts of *Isospora coerebae*, a coccidium species recovered from the bananaquit *Coereba flaveola* from Southeastern Brazil. Note the posterior refractile body (prb), crystalloid body (cb), inner (il) and outer (ol) layer of the oocyst wall, sporocyst residuum (sr) and the Stieda (sb) and sub-Stieda (ssb) bodies. Scale-bar = 10 µm.

Remarks: The oocysts recovered from the bananaquit in the current work are morphologically equivalent to the original description of *I. coerebae*, which have a typical and easily identifiable morphology, mainly in the Stieda and Sub-Stieda bodies (Berto *et al.* 2010). Table 1 compares the morphology and morphometry of the oocysts from the current work with those from the original description of *I. coerebae*. The typical morphology of the Stieda and Sub-Stieda bodies of *I. coerebae* are the basis of differentiation from another congeneric species, *Isospora cagasebi* Berto, Flausino, Luz, Ferreira and Lopes, 2008, which has the same host- and locality-types: *C. flaveola* in the Marambaia Island (Berto *et al.* 2008) (Table 1). However, in the current work no oocyst of *I. cagasebi* was observed, which, despite being similar in some characteristic features to *I. coerebae*, their sporocysts have knob-like Stieda body and prominent Sub-Stieda body, while *I. coerebae* has rounded Stieda body and short and wide Sub-Stieda body (Berto *et al.* 2010).

Phylogenetic analysis: Phylogenetic analysis included sequences from coccidians available in GenBank (Figs. 2; 3). *Toxoplasma gondii* (Nicolle and Manceaux, 1908) was used as the outgroup. In the phylogenetic analysis based on the COI1 gene (Fig. 2), *I. coerebae* was recovered in a clade with the highest similarity of 99% with *Isospora sepetibensis* Berto, Flausino, Luz, Ferreira and Lopes, 2008 from black-goggled tanagers *Trichothraupis melanops* (Vieillot, 1818) of the Itatiaia National Park, which is located in the mountainous region in the interior of Southeastern Brazil. In the COI2 based phylogenetic analysis (Fig. 3), *I. coerebae* sat in a large clade with *Isospora* spp. from passerines of North America, Europe and Asia, with the highest similarity of 98% with *Isospora greineri* Hafeez, Stasiak, Delnatte, El-Sherry, Smith and Barta, 2014 from superb starlings *Lamprotornis superbus* Rüppell, 1845 at the Toronto Zoo, Toronto, Canada.

TABLE 1. Comparative morphology of *Isospora* spp. recorded from *Coereba flaveola* of different localities in the Southeastern Brazil.

Coccidia	Hosts	Locality	References	Oocysts							
				Shape	Length (µm)	Width (µm)	L/W ratio	Wall	Residuum	Micropyle	Polar granule
***Isospora coerebae* Berto, Flausino, Luz, Ferreira & Lopes, 2010**	***Coereba flaveola***	Marambaia Island	Berto *et al.* (2010)	sub-spherical	23–27 (24.7)	21–26 (23.3)	1.0–1.1 (1.1)	smooth	absent	absent	absent
		Private Natural Heritage Reserve of Porangaba	**current work**	**sub-spherical**	**22–27 (24.4)**	**22–25 (22.9)**	**1.0–1.1 (1.07)**	**smooth**	**absent**	**absent**	**absent**
Isospora cagasebi Berto, Flausino, Luz, Ferreira & Lopes, 2008	*C. flaveola*	Marambaia Island	Berto *et al.* (2010)	sub-spherical	23–27 (25.2)	23–25 (24.5)	1.0–1.1 (1.1)	smooth	absent	absent	absent

Continued.

Coccidia	Hosts	Locality	References	Sporocysts						
				Shape	Length (µm)	Width (µm)	L/W ratio	Stieda body	Substieda body	Residuum
***Isospora coerebae* Berto, Flausino, Luz, Ferreira & Lopes, 2010**	***Coereba flaveola***	Marambaia Island	Berto *et al.* (2010)	elongate ovoidal	16–19 (17.7)	10–12 (10.9)	1.5–1.8 (1.6)	prominent and rounded	short and wide	present, scattered granules
		Private Natural Heritage Reserve of Porangaba	**current work**	**elongate ovoidal**	**16–19 (17.6)**	**10–11 (10.5)**	**1.6–1.8 (1.67)**	**prominent and rounded**	**short and wide**	present, scattered granules
Isospora cagasebi Berto, Flausino, Luz, Ferreira & Lopes, 2008	*C. flaveola*	Marambaia Island	Berto *et al.* (2010)	elongate ovoidal or ellipsoidal	17–20 (18.7)	10–12 (11.4)	1.5–1.7 (1.6)	knob-like	prominent	present, scattered granules

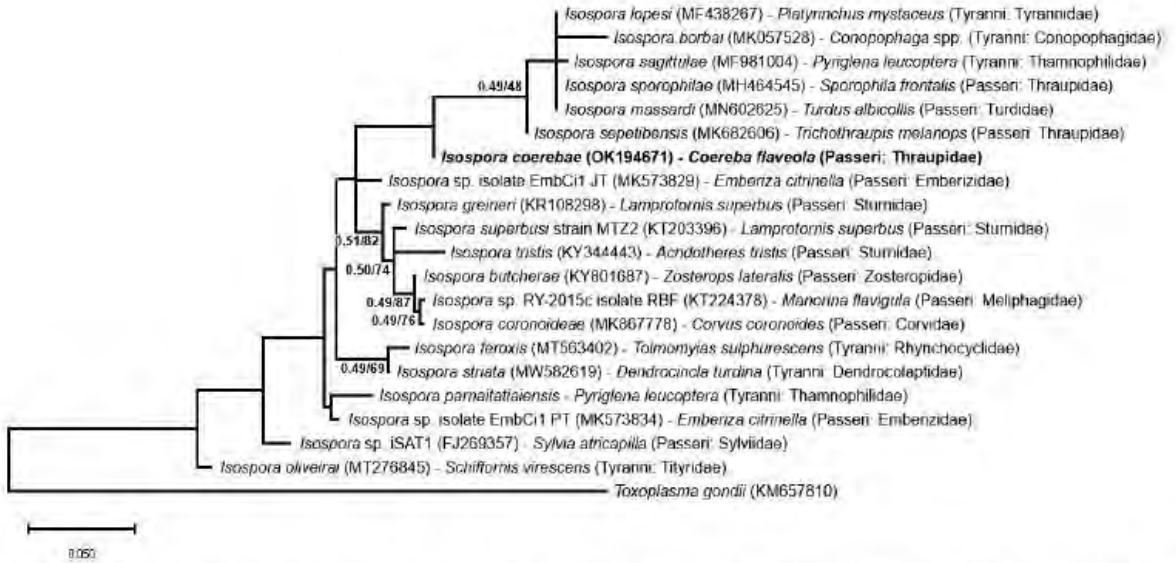

FIGURE 2. Maximum likelihood tree estimated from the COI1 gene sequences of coccidian species. Numbers at the nodes show posterior probabilities under the Bayesian Inference analysis/bootstrap values derived from Maximum Likelihood analysis. Scale bar represents the number of nucleotide substitutions per site.

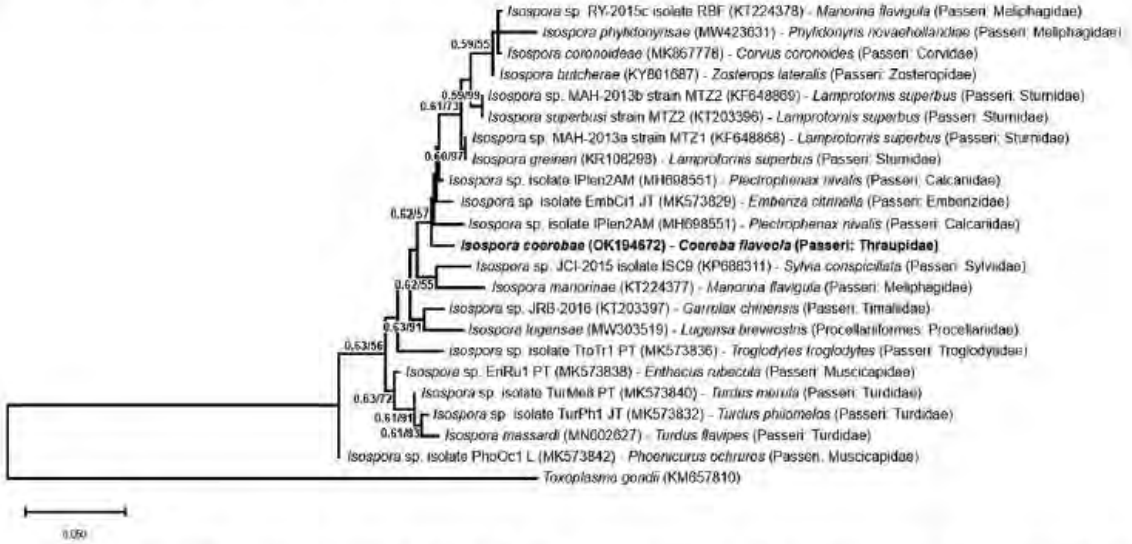

FIGURE 3. Maximum likelihood tree estimated from the COI2 gene sequences of coccidian species. Numbers at the nodes show posterior probabilities under the Bayesian Inference analysis/bootstrap values derived from Maximum Likelihood analysis. Scale bar represents the number of nucleotide substitutions per site.

Discussion

The molecular approach to the study of coccidia still has many inconsistencies, requiring more sequencing of different genes e genic regions, and in a larger number of species. The overwhelming majority of coccidian species described in the scientific literature were based on the morphological and morphometric study of oocysts. This is justified by the fact that molecular techniques are a relatively recent advent, whereas eimerid coccidians have been described since the 19th century (Duszynski & Wilber 1997; Tenter *et al.* 2002; Berto *et al.* 2014). Thus, many recent works show a molecular complementation, and consequently a phylogenetic analysis, of species already known to science, such as the contributions of Genovez-Oliveira *et al.* (2019); Genovez-Oliveira *et al.* (2020); Ortúzar-Fer-

reira *et al.* (2021) and Oliveira *et al.* (2021). Other works have even promoted taxonomic changes based on genetic information, such as the taxonomic rearrangement of *Cryptosporidium* (Barta & Thompson 2006).

According to Barta (2001) molecular tools should be useful, mainly to infer evolutionary relationships in protist parasites, considering that the morphological characteristics become limited in certain groups to distinguish species, even more for the understanding of evolutionary history. Adaptive irradiations and evolutionary convergences are phenomena that explain the diversity of living beings, but that can also become confusing or conflicting without a genetic approach (Wilson 1995). For this reason, molecular studies have been widely applied in recent decades, not as a substitute technique, but to complement the taxonomic and phylogenetic data of the species. In this sense, it is noteworthy that the morphology associated with the biological and ecological aspects of parasites and hosts continue to be elementary for any taxonomic and/or phylogenetic study. Molecular studies are, therefore, an exceptional complement to confirm or reject hypotheses, delineate more precisely the phylogeny of groups (monophylies, paraphylies, etc.), understand inherent evolutionary aspects (polymorphism and genetic variation within the same species) and support the description of new species (Hewitt *et al.* 1991, Godfray & Knapp 2004, Dolnik et *al.* 2009).

The genes commonly used in the sequencing of *Isospora* spp. are the ribosomal and mitochondrial genes, with 18s being one of the most widely used. While ribosomal genes are more conserved, mitochondrial genes accumulate more changes over time and are therefore less conserved. In this sense, mitochondrial genes allow for a better approach to species separation/identification and to delimit recent speciation events (Schrenzel *et al.* 2005; Dolnik *et al.* 2009; Ogedengbe *et al.* 2018). Among the mitochondrial genes sequenced for the study of *Isospora* spp., COI has been widely used, although recent work has sequenced other mitochondrial genes aiming at the complete mitochondrial genome (Ogedengbe *et al.* 2016; Hafeez *et al.* 2017; Yang *et al.* 2017).

In the current work, amplification/sequencing of a genic region of the 18S has been attempted, but has not been successful; in contrast, two genic regions of the COI were amplified/sequenced. The genic region named here as COI1 is pioneer for *Isospora* spp. from passerines (Dolnik *et al.* 2019) and has been widely used in the studies with molecular identification of coccidia of passerine birds. However, this small region of ~250 bp (COI1) has been shown to be unsuitable for phylogenetic studies, as it has not generated clades associated with the morphology, biology and/or ecology of the species (Oliveira *et al.* 2021). In this sense, the current work also used primers that amplify a larger genic region of COI, with ~650 bp (COI2), which was designed by Genovez-Oliveira *et al.* (2020). Although there is a small overlap between the COI1 and COI2 genic regions, the longer COI2 sequence should generate better phylogenetic results, although the very small number of species sequenced for this genic region does not allow for further conclusions. Therefore, observing the phylogenetic tree for the COI2 genic region (Figure 3), it is observed that *I. coerebae* was close to *Isospora* spp. from passerines from outside the Neotropical region, which were extensively sequenced for mitochondrial genes or had the mitochondrial genome completed, with only *I. massardi* Lopes, Berto, Luz, Galvão, Ferreira and Lopes, 2014 sequenced from thrushes *Turdus* spp. in Brazil for this same genic region. On the other hand, in the phylogenetic tree for COI1 (Figure 2), there is a greater amount of sequences of *Isospora* spp. from neotropical birds, including from the same Thraupidae family, such as the black-goggled tanager, which its parasite *I. sepetibensis* was the closest to *I. coerebae* for the COI1 genic region. However, the phylogenetic tree for COI1 (Figure 2) is still inconclusive, since coccidian species with close ecological, biological and morphological characteristics were distant and/or sat in distinct clades.

The identification of *I. coerebae* in the RPPN of Porangaba reinforces the possibility of the dispersion of coccidians from Marambaia Island to the mainland. In the work of Rodrigues *et al.* (2017), a coccidian originally described from passerines of the Marambaia Island was also identified in passerines in the mainland. In fact, the island has a sand zone of around 40 km in extension (Marambaia Coastal Restinga) connected to the mainland, which probably allows the migration of birds from the mainland to the island, favoring the dispersal of their coccidia.

Finally, based on the morphological and molecular features described above, *I. coerebae* is reported in the current work, documenting a new locality in the Brazilian Atlantic forest, the RPPN of Porangaba, Southeastern Brazil.

Acknowledgements

We are thankful to staff at the Private Natural Heritage Reserve Porangaba, mainly to the the owner Dr. Jürgen

Döbereiner (*in memorian*) and the environmental manager Luiz Fernado Cardoso, that allowed us to carry out data collections at the RPPN and use some facilities during the expeditions, as well as the fundamental help of the naturalist photographer José Felipe Pereira during the collections. This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ). CNO-F has a scholarship from CNPq/UFRRJ (Grant/Award Number: PVBS2485-2021). LASA, JLG-O and MSO has a scholarships from CAPES (Grant/Award Number: 001). BPB has a fellowship from CNPq (Grant/Award Number: 303899/2019-0) and from FAPERJ (Grant/Award Number: E-26/202.797/2019).

References

Barta, J.R. (2001) Molecular approaches for inferring evolutionary relationships among protistan parasites. *Veterinary Parasitology*, 101, 175–186.
https://doi.org/10.1016/S0304-4017(01)00564-7

Barta, J.R. & Thompson, R.C.A. (2006) What is *Cryptosporidium?* Reappraising its biology and phylogenetic affinities. *Trends in Parasitology*, 22 (10), 463–468.
https://doi.org/10.1016/j.pt.2006.08.001

Berto, B.P., Flausino, W., Luz, H.R., Ferreira, I. & Lopes, C.W.G. (2010) *Isospora coerebae* n. sp. (Apicomplexa: Eimeriidae) from the bananaquit Coereba flaveola (Passeriformes: Coerebidae) in South America. *Revista Brasileira de Parasitologia Veterinária*, 20 (1), 22-26.
https://doi.org/10.1590/S1984-29612011000100005

Berto, B.P., Flausino, W., McIntosh, D., Teixeira-Filho, W.L. & Lopes, C.W.G. (2011) Coccidia of New World passerine birds (Aves: Passeriformes): a review of Eimeria Schneider, 1875 and Isospora Schneider, 1881 (Apicomplexa: Eimeriidae). *Systematic Parasitology*, 80, 159–204.
https://doi.org/10.1007/s11230-011-9317-8

Berto, B.P. & Lopes, C.W.G. (2013) Distribution and Dispersion of Coccidia in Wild Passerines of the Americas. *In*: Ruiz, L. & Iglesias, F. (Eds.), *Birds: Evolution and Behavior, Breeding Strategies, Migration and Spread of Disease.* Nova Science Publishers, New York, New York, pp. 47–66.

Berto, B.P., McIntosh, D. & Lopes, C.W.G (2014) Studies on coccidian oocysts (Apicomplexa: Eucoccidiorida). *Revista Brasileira de Parasitologia Veterinária*, 23 (1), 1–15.
https://doi.org/10.1590/S1984-29612014001

Chapman, H.D., Barta, J.R., Blake, D., Gruber, A., Jenkins, M., Smith, N.C., Suo, X. & Tomley, F.M. (2013) Uma revisão seletiva dos avanços na pesquisa da coccidiose. *Advances in Parasitology*, 93–171.
https://doi.org/10.1016 / b978-0-12-407705-8.00002-1

Dolnik, O.V., Palinauskas, V. & Bensch S. (2009) Individual oocysts of *Isospora* (Apicomplexa: Coccidia) parasites from avian feces: from photo to sequence. *Journal of Parasitology*, 95, 169–174.
https://doi.org/10.1645/GE-1873.1

Duszynski, D.W. & Wilber, P. (1997) A guideline for the preparation of species descriptions in the Eimeridae. *Journal of Parasitology*, 83 (2), 333–336.
https://doi.org/10.2307/3284470

Fayer, R. (1980) Epidemiology of protozoan infection: the Coccidia. *Veterinary Parasitology*, 6, 75–103.
https://doi.org/10.1016/0304-4017(80)90039-4

Genovez-Oliveira, J.L., Cardozo, S.V., Oliveira, A.A., Lima, V.M., Ferreira, I. & Berto, B.P. (2019) Morphological and molecular identification of *Isospora sepetibensis* (Chromista: Miozoa: Eimeriidae) from a new host, *Trichothraupis melanops* (Passeriformes: Thraupidae: Tachyphoninae) in South America. *Acta protozoologia*, 58, 17–23.
https://doi.org/10.4467/16890027AP.19.007.10838

Genovez-Oliveira, J.L., Oliveira, M.S., Thode-Filho, S., Cardozo, S.V., Oliveira, A.A., Lima, V.M., Ferreira, I. & Berto, B.P. (2020) Morphological and molecular identification of *Isospora massardi* Lopes, Berto, Luz, Galvão, Ferreira & Lopes, 2014 (Chromista: Miozoa: Eimeriidae) from thrushes *Turdus* spp. (Passeriformes: Turdidae) in South America. *Parasitology Internacional*, 75, 102040.
https://doi.org/10.1016/j.parint.2019.102040

Ghimire, T.R. (2010) Redescription of genera of family Eimeriidae Minchin, 1903. *International Journal of Life Sciences*, 4, 26–47.
https://doi.org/10.3126/ijls.v4i0.3285

Godfray, H.C.J. & Knapp, S. (2004) Introduction. Taxonomy for the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 359 (1444), 559-569.
https://doi.org/10.1098/rstb.2003.1457

Hafeez, M.A. & Barta, J.R. (2017) The complete mitochondrial genome sequences of two *Isospora* species (Eimeriidae, Eu-

coccidiorida, Coccidiasina, Apicomplexa) causing coccidiosis in superb glossy starlings, Lamprotornis superbus (Aves: Sturnidae). *Mitochondrial DNA Part B*, 2 (2), 895–896.
https://doi.org/10.1080/23802359.2017.1407698

Hewitt, G.M., Johnston, A.W.B. & Joung, J.P.W. (1991) *Molecular Techniques in Taxonomy*. Springer-Verlag, Berlin and New York, 410 pp.

Júnior, A.B., Silva, E.N., Fábio, J.D., Sesti, L. & Zuanaze, M.A.F. (2009) *Doença das aves. Segunda Edição*. FACTA—Fundação APINCO de Ciência e Tecnologia Avícolas, Campinas-SP, 1577 pp.

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, 35, 1547–1549.
https://doi.org/10.1093/molbev/msy096

Levine, N.D. (1985) *Veterinary Protozoology. 1ª Edition*. Iowa State University Press, Ames, Iowa, 414 pp.

Ogedengbe, J.D., Hanner, R.H. & Barta, J.R. (2011) DNA barcoding identifies *Eimeria* species and contributes to the phylogenetics of coccidian parasites (Eimeriorina, Apicomplexa, Alveolata). *International Journal for Parasitology*, 41, 843–850.
https://doi.org/10.1016/j.ijpara.2011.03.007

Ogedengbe, M.E., Brash, M. & Barta, J.R. (2016) The complete mitochondrial genome sequence of an *Isospora* sp.(Eimeriidae, Eucoccidiorida, Coccidiasina, Apicomplexa) causing systemic coccidiosis in domestic Canaries (*Serinus canaria* Linn.). *Mitochondrial DNA Part A*, 27 (5), 3315–3317.
https://doi.org/10.3109/19401736.2015.1018201

Ogedengbe, M.E., El-Sherry, S., Ogedengbe, J.D., Chapman, H.D. & Barta, J.R. (2018) Phylogenies based on combined mitochondrial and nuclear sequences conflict with morphologically defined genera in the eimeriid coccidia (Apicomplexa). *International Journal for Parasitology*, 48, 59–69.
https://doi.org/10.1016/j.ijpara.2017.07.008

Oliveira, M.S., Mello, E.R., Cardozo, S.V., Oliveira, A.A., Lima, V.M., Ferreira, I., Berto, B.P. (2021) Distribution, redescription and molecular identification of *Isospora striata* McQuistion et. al 1997 (Eimeriidae) from woodcreepers (Dendrocolaptidae) in South America. *Parasitology Research*, 120, 2585–2593.
https://doi.org/10.1007/s00436-021-07140-9

Ortúzar-Ferreira, C.N., Mello, E.R., Melo, J.O., Oliveira, M.S., Thode-Filho, S., Cardozo, S.V., Oliveira, A.A., Lima, V.M., Ferreira, I. & Berto, B.P. (2021) Redescription and the molecular identification of *Isospora feroxis* Berto, Luz, Flausino, Ferreira & Lopes, 2009 (Eimeriidae) from tyrant-flycatchers (Tyrannoidea) in South America. *Systematic Parasitology*, 98, 333–341.
https://doi.org/10.1007/s11230-021-09981-5

Rodrigues, M.B., de Pinho, I.F., da Silva, L.M., doBomfim Lopes, B., Luz, H.R., Ferreira, I., Lopes, C.W.G. & Berto, B.P. (2017) The ruby-crowned tanager *Tachyphonus coronatus* Vieillot, 1822 (Passeriformes: Thraupidae) as a new host for *Isospora ramphoceli* Berto, Flausino, Luz, Ferreira, Lopes, 2010 in Brazil. *Acta parasitologica*, 62 (2), 306–311.
https://doi.org/10.1515/ap-2017-0038

Schrenzel, M.D., Maalouf, G.A., Gaffney, P.M., Tokarz, D., Keener, L.L., McClure, D., Griffey, S., McAloose, D. & Rideout, B.A. (2005) Molecular characterization of isosporoid coccidia (*Isospora and Atoxoplasma* spp.) in passerine birds. *The Journal of Parasitology*, 91 (3), 635–647.
https://doi.org/10.1645/GE-3310

Sigrist, T. (2014) *Guia de Campo: Avifauna Brasileira*. Avis Brasilis, São Paulo, 608 pp.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, 24 (8), 1596–1599.
https://doi.org/10.1093/molbev/msm092

Tenter, A., Barta, J.R., Beveridge, I., Duszynski, D.W., Mehlhorn, H., Morrison, D.A., Thompson, R.C.A. & Conrad, P. (2002) The conceptual basis for a new classification of the coccidia. *International Journal of Parasitology*, 32, 505–616.
https://doi.org/10.1016/S0020-7519(02)00021-8

Wilson, K.H. (1995) Molecular biology as a tool for taxonomy. *Clinical Infectious Diseases*, 20 (Supplement 2), S117–S121.
https://doi.org/10.1093/clinids/20.Supplement_2.S117

Yang, R., Brice, B., Elloit, A., Lee, E. & Ryan, U. (2014) Morphological and molecular characterization of *Eimeria paludosa* coccidian parasite (Apicomplexa: Eimeriidae) in a dusky moorhen (Gallinula tenebrosa, Gould, 1846) in Australia. *Experimental Parasitology*, 147, 16–22.
https://doi.org/10.1016/j.exppara.2014.10.010

Yang, R., Brice, B., Elliot, A. & Ryan, U. (2015) *Isospora serinuse* n. sp. (Apicomplexa: Eimeriidae) from a domestic canary (*Serinus canaria* forma domestica) (Passeriformes: Fringillidae) in Western Australia. *Experimental Parasitology*, 159, 59–66.
https://doi.org/10.1016/j.exppara.2015.08.020

Yang, R., Brice, B., Oskam, C., Zhang, Y., Brigg, F., Berryman, D. & Ryan, U. (2017) Characterization of two complete *Isospora* mitochondrial genomes from passerine birds: *Isospora serinuse* in a domestic canary and *Isospora manorinae* in a yellow-throated miner. *Veterinary Parasitology*, 237, 137–142.
https://doi.org/10.1016/j.vetpar.2017.01.027

Syst Parasitol (2022) 99:285–297
https://doi.org/10.1007/s11230-022-10025-9

Isospora spp. (Eimeriidae) from green-winged saltators *Saltator similis* d'Orbigny & Lafresnaye, 1837 (Thraupidae) from captivity near the Conservation Unit of the Itatiaia National Park in Southeastern Brazil

Carla Maronezi · Mariana de Souza Oliveira · Jhon Lennon Genovez-Oliveira · Ericson Ramos de Mello · Patrícia Barizon Cepeda · Águida Aparecida de Oliveira · Viviane Moreira de Lima · Bruno Pereira Berto

Received: 1 November 2021 / Accepted: 14 January 2022 / Published online: 3 February 2022

Abstract The present study identifies three *Isospora* spp. recorded from faecal samples of green-winged saltators *Saltator similis* d'Orbigny & Lafresnaye kept in captivity in the surroundings of the Itatiaia National Park, which is a Conservation Unit with a high degree of vulnerability located in the Serra da Mantiqueira on the border of the States of Rio de Janeiro and Minas Gerais. *Isospora saltatori* Berto, Balthazar, Flausino & Lopes, 2008, *Isospora trincaferri* Berto, Balthazar, Flausino & Lopes, 2008 and *Isospora similisi* Coelho, Berto, Neves, Oliveira, Flausino & Lopes, 2013 were compatible in all characteristic features with their respective original descriptions, despite some divergences that are discussed in this study. In addition to the preliminary morphological identification, this study provided a preliminary genotypic identification of these three *Isospora* spp. via sequencing of the mitochondrial cytochrome *c* oxidase subunit 1 gene, which was suitable for the genotypic differentiation of these three coccidians, but was inconclusive in the phylogenetic analysis. Finally, this study discusses the environmental risks of these coccidians in captivity of green-winged saltators in the surroundings of the Itatiaia National Park.

C. Maronezi
Programa de Pós-Graduação em Ciência, Tecnologia e Inovação em Agropecuária, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7, Seropédica, Rio de Janeiro 23897-000, Brazil

M. S. Oliveira · J. L. Genovez-Oliveira
Programa de Pós-Graduação em Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7, Seropédica, Rio de Janeiro 23897-000, Brazil

E. R. de Mello
Programa de Pós-Graduação em Ciências Veterinárias, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7, Seropédica, Rio de Janeiro 23897-000, Brazil

P. B. Cepeda
Centro Universitário de Barra Mansa, Rua Vereador Pinho de Carvalho 267, Barra Mansa, Rio de Janeiro 27330-550, Brazil

Á. A. de Oliveira
Departamento de Microbiologia e Imunologia Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7, Seropédica, Rio de Janeiro 23897-000, Brazil

V. M. de Lima · B. P. Berto (✉)
Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7, Seropédica, Rio de Janeiro 23897-000, Brazil
e-mail: bertobp@ufrrj.br

Introduction

The Itatiaia National Park (Parque Nacional do Itatiaia) is located in Serra da Mantiqueira, covering the territory of the Municipalities of Itatiaia, Resende, Itamonte and Bocaina de Minas in the States of Rio de Janeiro and Minas Gerais, with altitudes ranging between 540m and 2,791m at Pico das Agulhas Negras, the highest point of the State of Rio de Janeiro and 5th highest in Brazil. This was the first National Park in Brazil, constituted on June 14, 1937, as an Integral Protection Conservation Unit (ICMBIO, 2016).

The green-winged saltators *Saltator similis* d'Orbigny & Lafresnaye are passerines of the family Thraupidae and subfamily Saltatorinae, which are part of the bird community that occurs around and within the Itatiaia National Park (Maia-Gouvêa et al., 2005; BirdLife International, 2021). This species is one of the most appreciated wild birds in Brazil, especially for its vocalization, being considered one of the favorite passerines for breeding in captivity, due to a cultural context established by Brazilians, who keep wild songbirds in cages (Sick, 1997). In this sense, green-winged saltators are classified among the main passerines seized from the illegal wildlife trafficking (biopiracy), by environmental inspection (Destro et al., 2012).

The confinement in which the passerines are submitted in the biopiracy cause constant stress that favors the occurrence of illnesses (Maia-Gouvêa et al., 2005; Berto & Lopes, 2020). Among the diseases that affect passerines in captivity are nutritional disorders, viral, bacterial, fungal and parasitic diseases (Coelho et al., 2012); however, parasitic diseases are the most frequent, which can range from subclinical to deadly (Freitas, 2002). Among the parasites of importance in passerines are the coccidians, which are intestinal protozoans that can cause reduced weight gain, affect the intestinal reabsorption of nutrients, reduce the fertility of birds and even lead to death, being frequently observed in faecal samples (Oliveira, 2017).

In this context, the present study identifies three *Isospora* spp. recorded from *S. similis*, from faecal samples from green-winged saltators kept in captivity in the surroundings of the Itatiaia National Park, recording new localities and highlighting the possibility of occasional transmissions or even epizootics in populations of *S. similis* and other free-living Saltatorinae of the Itatiaia National Park. Additionally, this study provides a preliminary genotypic identification of these three *Isospora* spp. via sequencing of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene.

Materials and methods

Sample collection

Faecal samples were collected from eight specimens of *S. similis* which were kept alone in cages from five different breeders of green-winged saltators located near the territory of the Itatiaia National Park (22°30'S, 44°34'W). The bottoms of the cages were previously covered with absorbent paper and faeces collected immediately after defecation. The collected faeces were then placed in centrifuge tubes containing a potassium dichromate 2.5% ($K_2Cr_2O_7$) solution at 1:6 (v/v).

Morphological analyses

Samples were taken to the Laboratório de Biologia de Coccídios, Universidade Federal Rural do Rio de Janeiro (UFRRJ). Samples were incubated at room temperature (25°C) for 10 days or until ~70% of the oöcysts were sporulated. Oöcysts were isolated by flotation in Sheather's sugar saturated solution (specific gravity: 1.20) and examined microscopically using the technique described by Duszynski and Wilber (1997) and Berto et al. (2014). Morphological observations, line drawings, photomicrographs and measurements were made using an Olympus BX binocular microscope (Olympus Optical, Tokyo, Japan) coupled to a digital camera Eurekam 5.0 (BEL Photonics, Monza, Italy). Line drawings were edited using Corel DRAW® and Corel PHOTO-PAINT (Corel Draw Graphics Suite, Version 2020, Corel Corporation, Canada). All measurements are in micrometres and are given as the range followed by the mean in parentheses. Linear regression was used to determine the distribution of oöcysts using methods proposed by Norton & Joyner (1981) and subsequently modified by Berto et al. (2014). The graphs and coefficient of regression line were obtained using the software Microsoft Excel 2013® (Microsoft, Redmond, Washington).

Molecular analyses

An individual oöcyst from each faecal sample of *S. similis* was isolated from serial dilutions of the oöcysts in drops on a microscope slide using a sterile micropipette. This isolated oöcyst was resuspended in PBS and washed by centrifuging until the supernatant became clear (Dolnik et al., 2009). DNA was extracted from the purified oöcysts using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, São Paulo, Brazil) according to the manufacturer's instructions. In order to fully lyse the oöcyst, four freeze-thaw cycles were applied prior to the DNA extraction. The PCR amplification for the COI gene was carried out using a nested PCR, as previously described by Dolnik et al. (2009) and Yang et al. (2015). The external primers COIbF1 (5'-GWT CAT TAG TAT GGG CAC ATC A-3') and COIbR1 (5'-CCA AGA GAT AAT ACR AAR TGG AA-3') produced a PCR product of 302 bp in size. The internal primes COIbF2 (5'-GGG CAC ATC ATA TGA TGA C-3') and COIbR2 (5'-ATA GTA TGT ATC ATG TAR WGC AA-3') produced an amplicon of 257 bp in size. The PCR reaction contained 12.5µL of GoTaq® G2 Hot Start Colorless Master Mix (Promega Labs) (1X), 0,25 µL of each Primer (0.2µM), 9µL of Nuclease Free Water, 3 µl of DNA (for primary reaction) or 3µl primary PCR product (for the secondary reaction). Both primary and secondary PCR were conducted using the same cycling conditions: 1 cycle of 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 47°C for 45 s, and 72°C for 1 min and a final extension of 72°C for 5 min. The amplicons from the second round of PCR were purified using the Qiagen MinElute PCR Purification (Qiagen, São Paulo, Brazil).

DNA sequence analyses

All PCR products were sequenced using the PCR forward and reverse primers by Ludwig Biotechnology, were an ABI-Prism 3500 Genetic Analyzer (Applied Biosystems, Foster City, California) was used for Sanger sequencing. The results of the sequencing reactions were analyzed and edited using the program Chromas 2.6. Sequences were compared with other coccidian parasites available on the GenBank database using the Basic Local Alignment Search Tool (BLAST). Phylogenetic trees were constructed for coccidian species at the COI sequences aligned with additional isolates from GenBank. Distance analyses and phylogenies were conducted using MEGA X (Kumar et al. 2018). Briefly, Sanger sequencing chromatogram files were imported into MEGA X and the nucleotide sequences of each genic region was curated, analysed, and aligned with reference sequences from GenBank using Clustal W (http://www.clustalw.genome.jp). Maximum likelihood (ML) and Neighbor-Joining (NJ) trees were constructed and the distances were computed using the Tamura-Nei method based on model selection using ModelTest in MEGA X. Bootstrap analyses were conducted using 1,000 replicates to assess the reliability of inferred tree topologies.

Results

Eight *S. similis* were examined and seven of them (88%) were passing coccidia. Three distinct morphotypes were observed and morphologically identified as *Isospora saltatori* Berto et al., 2008, *Isospora trincaferri* Berto et al., 2008 and *Isospora similisi* Coelho et al., 2013 (Berto et al. 2008). The morphologies of these oöcysts are specifically reported below.

Eimeriidae Minchin, 1903
Isospora Schneider, 1881

***Isospora saltatori* Berto et al., 2008**

Host: *Saltator similis* d'Orbigny & Lafresnaye, 1837 (Aves: Passeriformes: Thraupidae: Saltatorinae) green-winged saltator.

Locality: Five captive breeding colonies of green-winged saltators in the surroundings of the Itatiaia National Park (22°30'S, 44°34'W), Southeastern Brazil.

Specimens: Photomicrographs are deposited and available (http://r1.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under repository number 116/2021. Photovouchers of the host specimens are deposited in the same collection.

Site in host: Unknown; oöcysts recovered from faeces.

Prevalence: 75% (6/8).

Representative DNA sequence: Representative COI sequences were deposited in the GenBank database under the accession number OK631535.

Morphology (Fig. 1A-E)

Sporulated oöcyst

Oöcysts (n = 56) subspheroidal, 16–21 × 16–20 (18.7 × 18.0); length/width (L/W) ratio 1.0–1.1 (1.04). Wall bi-layered, 1.1–1.7 (1.4) thick, outer layer smooth, *c.*2/3 of total thickness. Micropyle, oöcyst residuum and polar granule absent.

Sporocyst and sporozoites

Sporocysts (n = 56) 2, ovoidal to ellipsoidal, 12–15 × 7–10 (13.6 × 8.5); L/W ratio 1.4–1.8 (1.61). Stieda body present, flattened to half-moon-shaped, 0.4–0.6 × 1.2–1.6 (0.5 × 1.4); sub-Stieda body present, rounded, 0.8–1.2 × 1.6–1.8 (1.0 × 1.7); parastieda body absent; sporocyst residuum present, composed of granules of different sizes slightly clustered, but also scattered among sporozoites. Sporozoites 4, with a robust posterior refractile body, but nucleus is indiscernible.

***Isospora trincaferri* Berto et al., 2008**

Host: *Saltator similis* d'Orbigny & Lafresnaye, 1837 (Aves: Passeriformes: Thraupidae: Saltatorinae) green-winged saltator.

Locality: Five captive breeding colonies of green-winged saltators in the surroundings of the Itatiaia National Park (22°30'S, 44°34'W), Southeastern Brazil.

Specimens: Photomicrographs are deposited and available (http://rl.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under repository number 117/2021. Photovouchers of the host specimens are deposited in the same collection.

Site in host: Unknown; oöcysts recovered from faeces.

Prevalence: 38% (3/8).

Representative DNA sequence: Representative COI sequences were deposited in the GenBank database under the accession number OK631536.

Morphology (Fig. 2A-E)

Sporulated oöcyst

Oöcysts (n = 44) subspheroidal to ovoidal, 20–26 × 19–24 (21.4 × 19.8); length/width (L/W) ratio 1.0–1.2 (1.08). Wall bi-layered, 1.2–1.8 (1.6) thick, outer layer smooth, c.2/3 of total thickness. Micropyle inconspicuous, being slightly observed in only a few oöcysts. Oöcyst residuum absent, but 1–2 (usually only 1) polar granules are present.

Sporocyst and sporozoites

Sporocysts (n = 44) 2, ovoidal to ellipsoidal, 14–19 × 8–12 (15.6 × 9.7); L/W ratio 1.4–1.9 (1.61). Stieda body present, knob-like to bubble-shaped, 1.1–1.4 × 2.0–2.8 (1.3 × 2.3); sub-Stieda body present, rounded to trapezoidal, 1.8–2.5 × 3.0–3.6 (2.1 × 3.4); parastieda body absent; sporocyst residuum present, usually as a distinctly subspheroidal to ellipsoidal body consisting of numerous small granules that appear to be membrane-bounded, 5.3–7.4 × 4.7–5.3

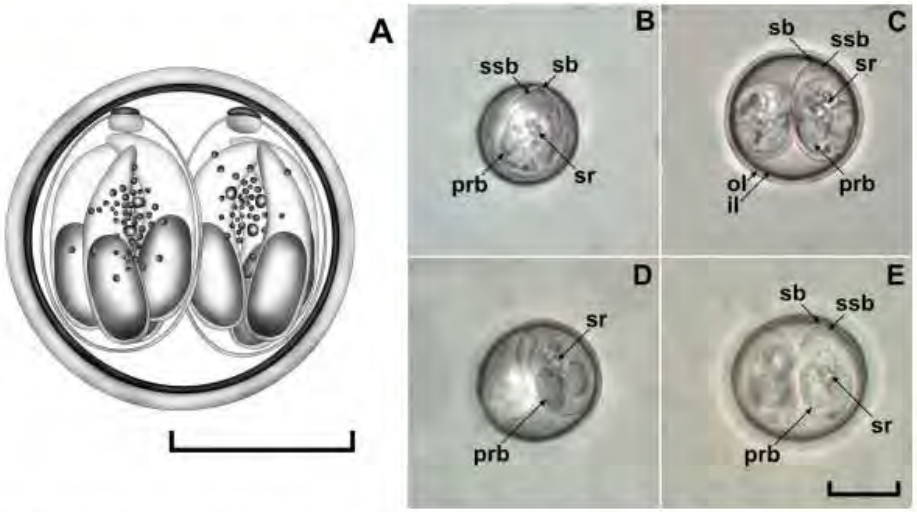

Fig. 1 Composite line drawing (A) and photomicrographs (B–E) of sporulated oöcysts of *Isospora saltatori* from green-winged saltators *Saltator similis*. Note the inner (il) and outer (ol) layers of the oöcyst wall; Stieda (sb) and sub-Stieda bodies (ssb); sporocyst residuum (sr); and posterior refractile body (prb). Scale-bar: 10 µm

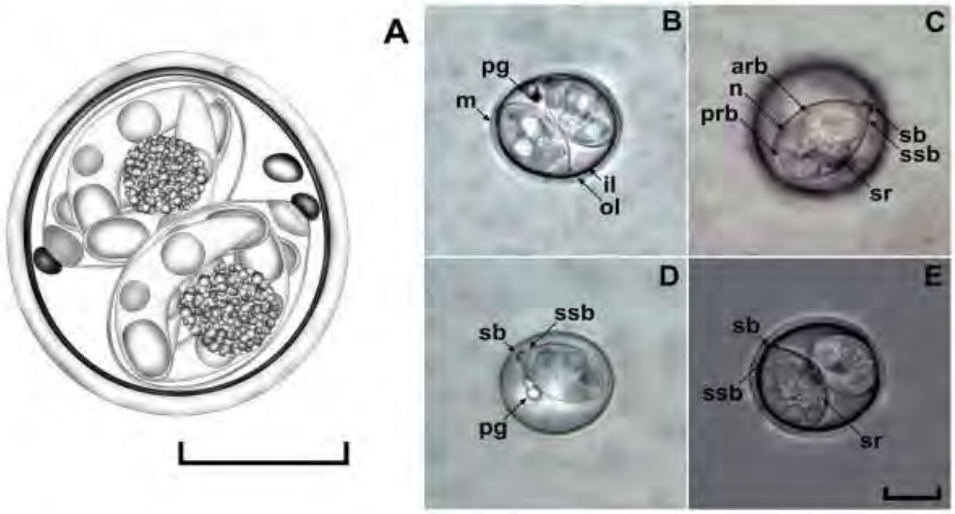

Fig. 2 Composite line drawing (A) and photomicrographs (B–E) of sporulated oöcysts of *Isospora trincaferri* from green-winged saltators *Saltator similis*. Note the inner (il) and outer (ol) layers of the oöcyst wall; micropyle (m); nucleus (n); polar granule (pg); Stieda (sb) and sub-Stieda bodies (ssb); sporocyst residuum (sr); anterior (arb) and posterior (prb) refractile bodies. Scale-bar: 10 µm

(5.9 × 5.0). Sporozoites 4, vermiform, with posterior and anterior refractile bodies and centrally located nucleus.

***Isospora similisi* Coelho et al., 2013**

Host: *Saltator similis* d'Orbigny & Lafresnaye, 1837 (Aves: Passeriformes: Thraupidae: Saltatorinae) green-winged saltator.

Locality: Five captive breeding colonies of green-winged saltators in the surroundings of the Itatiaia National Park (22°30'S, 44°34'W), Southeastern Brazil.

Specimens: Photomicrographs are deposited and available (http://rl.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under repository number 118/2021. Photovouchers of the host specimens are deposited in the same collection.

Site in host: Unknown; oöcysts recovered from faeces.

Prevalence: 38% (3/8).

Representative DNA sequence: Representative COI sequences were deposited in the GenBank database under the accession number OK631537.

Morphology (Fig. 3A-E)

Sporulated oöcyst

Oöcysts (n = 48) subspheroidal, 20–26 × 19–24 (23.3 × 22.0); length/width (L/W) ratio 1.0–1.1 (1.06). Wall bi-layered, 1.4–1.8 (1.7) thick, outer layer smooth, c.2/3 of total thickness. Micropyle and oöcyst residuum are absent, but splinter-like or comma-like polar granules are present.

Sporocyst and sporozoites

Sporocysts (n = 48) 2, ovoidal to slightly piriform, 15–18 × 10–12 (16.6 × 11.0); L/W ratio 1.4–1.7 (1.51). Stieda body present, half-moon-shaped to knob-like, 0.7–1.6 × 2.1–2.7 (1.0 × 2.4); sub-Stieda body present, rounded to trapezoidal, 2.3–3.4 × 3.3–4.8 (2.7 × 4.1), infrequently with density variations resembling a compartmentalized substieda; parastieda body absent; sporocyst residuum present, composed of spherules of different sizes. Sporozoites 4, vermiform, with posterior refractile body and centrally located nucleus.

Phylogenetic analysis

DNA amplification of the oöcyst of *I. saltatori*, *I. trincaferri* and *I. similisi* showed clear bands of *c.*250 bp. Phylogenetic analysis included 31 sequences for avian *Isospora* spp. available on GenBank (Fig. 4). *Toxoplasma gondii* (Nicolle & Manceaux, 1908) was used as the outgroup. The three *Isospora* spp. of the present study had different COI sequences from each other: *Isospora saltatori* was 91% similar with *I. trincaferri* and 93% with *I. similisi*; and *I. trincaferri* was 97% similar to *I. similisi*. These *Isospora* spp.

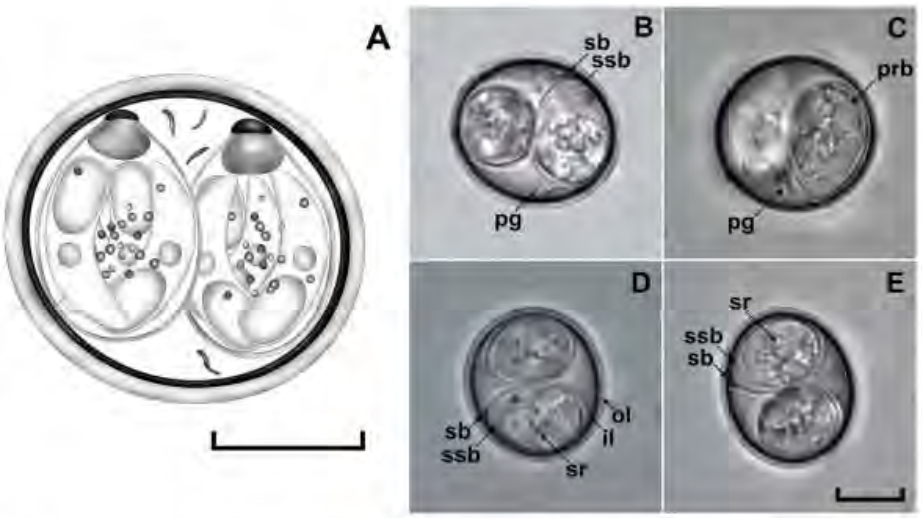

Fig. 3 Composite line drawing (A) and photomicrographs (B–E) of sporulated oöcysts of *Isospora similisi* from green-winged saltators *Saltator similis*. Note the inner (il) and outer (ol) layers of the oöcyst wall; polar granules (pg); Stieda (sb) and sub-Stieda bodies (ssb); sporocyst residuum (sr); posterior refractile body (prb). Scale-bar: 10 μm

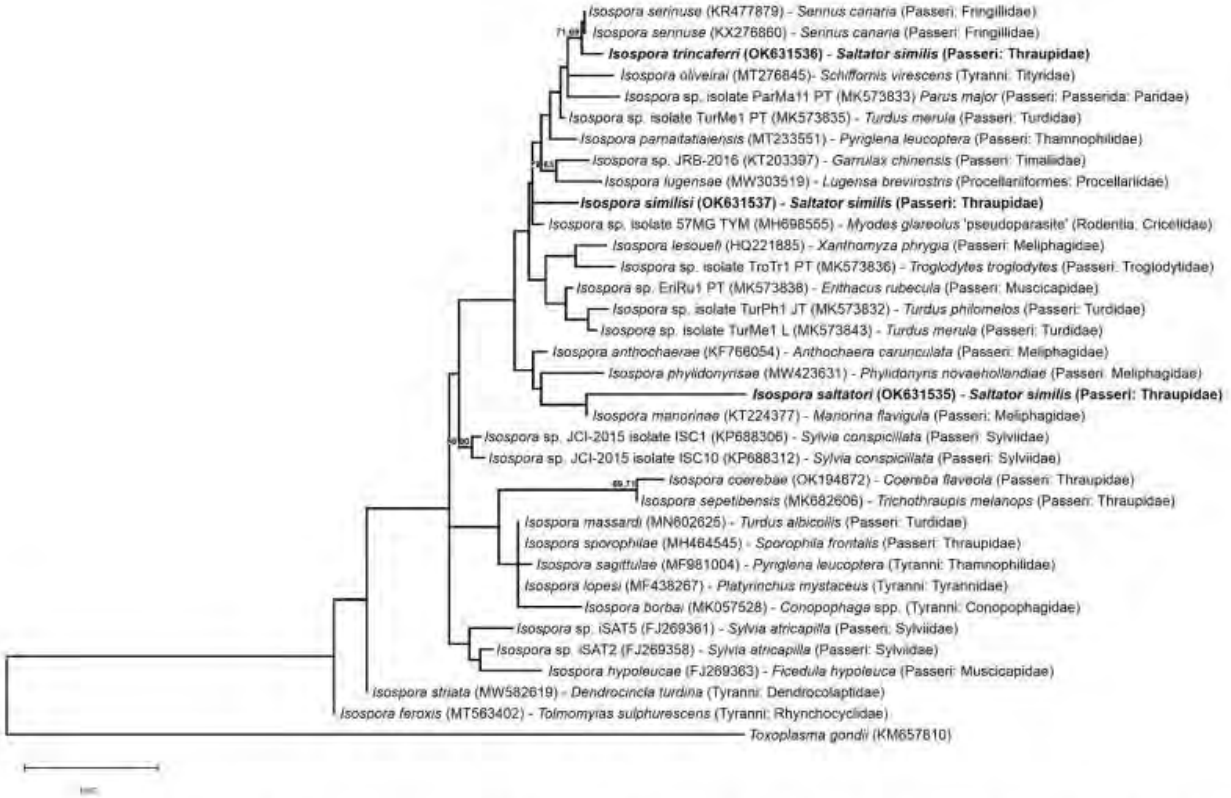

Fig. 4 Maximum likelihood tree estimated from the COI sequences. Numbers at nodes represent bootstrap support (1,000 replicates; only values > 50% shown) for Neighbor-Joining and Maximum Likelihood, respectively. The scale-bar represents the number of nucleotide substitutions per site.

from *S. similis* have been separated from each other in the phylogenetic analysis, into clades containing *Isospora* spp. from Neotropical passerines, but also from passerines of the Old World and Oceania. *Isospora trincaferri* and *I. similisi* have the highest similarities of 97% with *Isospora serinuse* Yang, Brice, Elliot et Ryan, 2015 from island canaries *Serinus canaria* (Linnaeus); while *I. saltatori* had the highest similarity of 94% with *Isospora manorinae* Yang, Brice, Jian & Ryan, 2016 from the yellow-throated miner *Manorina flavigula* (Gould).

Discussion

The three *Isospora* spp. reported in the present study were compatible in all characteristic features with their respective original descriptions, despite the large morphometric range observed in the oöcysts of the samples in this study, as well as related to morphometry from the original and/or previous descriptions (Table 1).

Isospora saltatori had the smallest morphometric range and the greatest similarity with previous reports. It is just important to emphasize that, in Barreto et al. (2020), oöcysts morphologically identifiable as *I. saltatori*, obtained from green-winged saltators from a rehabilitation center in Southeastern Brazil, were described as *Isospora ferri* Barreto, Vilela, Houri, Lara, Torres, Silva, Castro-Filho, Costa & Martins, 2020; however, the measurements and photomicrographs presented are fully compatible with *I. saltatori*, mainly due to the robust posterior refractile bodies of the sporozoites, which are typical of the species. Furthermore, these authors did not detail the differences that would justify a new species and did not present a line drawing.

Isospora trincaferri is the species with the most reports since its original description, including more than one host: The buff-throated saltator *Saltator maximus* (Statius Muller). However, even before the original description of this species, its oöcysts had already been observed, once that the report of *Isospora vanriperorum* (Levine, 1982) by Lopes et al. (2007) is indicated in this study as a misidentification. The photomicrograph and line drawing presented in Lopes et al. (2007) are morphologically compatible with *I. trincaferri* and, mainly, the identification of *I. vanriperorum* from *S. similis* in Brazil is quite unlikely for two reasons: (1) *Isospora vanriperorum* was described from northern cardinals *Cardinalis cardinalis* (Linnaeus) in North America, therefore this coccidian is recorded in the host family Cardinalidae, which is distinct from the family of saltators that are Thraupidae (Saltatorinae) (note that *S. similis* was classified as Cardinalidae in 2007); and (2) *C. cardinalis* has a geographic distribution restricted to North America in contrast to *Saltator* spp. that are strictly Neotropical, therefore transmissions from *C. cardinalis* to *S. similis* are unlikely, even considering introductions by illegal trafficking or for legalized commercial breeding.

The morphometry of *I. trincaferri* had a wide range, not only observing the oöcysts of the samples in this study, but also in relation to the measurements of the other studies (Table 1). This wide range of measurements must be a factor inherent to the coccidian species, but it may also result from environmental factors associated with stress, immunosuppression, etc. (Berto & Lopes, 2020). In fact, most reports of coccidians from *S. similis* were made from captive birds and/or birds recently recovered from illegal trafficking, therefore birds subjected to environmental factors that are known to influence the morphology of coccidian oöcysts (Berto & Lopes, 2020). It is also noteworthy that certain antibiotic and/or coccidiostatic drugs frequently used by bird breeders interfere in the sporogony process, resulting in the formation of morphologically and morphometrically altered and/or mutant oöcysts (Li et al. 2010). In this context, Barreto et al. (2020) misidentified *I. trincaferri* and described a new species, *Isospora beagai* Barreto, Vilela, Houri, Lara, Torres, Silva, Castro-Filho, Costa & Martins, 2020, from potentially altered oöcysts, but which are identifiable as *I. trincaferri* by the photomicrographs presented. Among these photomicrographs a mutant oöcyst with only one sporocyst is observed, which is a mutation commonly associated with birds treated with certain drugs and which may also have been the cause of the larger measurements of oöcysts by Barreto et al. (2020) (Table 1).

The oöcysts of *I. similisi* identified in the samples in this study had all the characteristic features of the original description by Coelho et al. (2013), which are quite typical, especially in relation to splinter-like or comma-like polar granules. However, they were smaller than those in the original description, confirming the statements by Berto & Lopes (2020), that the more stressed/immunosuppressed the host birds

Table 1 Comparative morphology of *Isospora* spp. recorded from saltators (Passeriformes: Thraupidae: Saltatorinae)

Species	Host	References	Oöcyst					
			Shape	Size (µm)	Shape index	Polar granule	Wall	Micropyle
Isospora pityli McQuistion, Capparella, 1992	*Saltator grossus* (Linnaeus)	McQuistion & Capparella (1992)	subspheroidal	20–21 × 17–20 (20.1 × 18.8)	1.0–1.2 (1.07)	absent	smooth, (1.5)	absent
Isospora formarum McQuistion, Capparella, 1992	*Saltator grossus* (Linnaeus)	McQuistion & Capparella (1992)	subspheroidal	21–27 × 20–25 (24.6 × 23.5)	1.0–1.1 (1.05)	absent	smooth, (1.5)	absent
Isospora saltatori Berto et al., 2008	*Saltator similis* d'Orbigny & Lafresnaye	Berto et al. (2008)	subspheroidal	17–20 × 16–20 (18.3 × 17.9)	1.0–1.1 (1.0)	absent	smooth, 1.0–1.2 (1.1)	absent
	S. similis	Barreto et al. (2020)[1]	subspheroidal	16–22 × 15–22 (20 × 18)	1.0–1.2 (1.0)	absent	smooth, (1.0)	absent
	S. similis	present study	subspheroidal	16–21 × 16–20 (18.7 × 18.0)	1.0–1.1 (1.04)	absent	smooth, 1.1–1.7 (1.4)	absent
Isospora trincaferri Berto et al., 2008	*S. similis*	Lopes et al. (2007)[2]	subspheroidal	19–26 × 18–26 (23.1 × 22.4)	(1.04)	present, 1	smooth	absent
	S. similis	Berto et al. (2008)	subspheroidal to ellipsoidal	24–29 × 22–25 (26.2 × 23.6)	1.0–1.2 (1.1)	present, 1	smooth, 1.0–1.3 (1.2)	absent
	Saltator maximus Müller	Lopes et al. (2013)	subspheroidal to ellipsoidal	24–27 × 23–26 (25.0 × 24.0)	1.0–1.1 (1.04)	present, 1–2	smooth, (1.2)	absent
	S. similis	Barreto et al. (2020)[3]	ovoidal	17–32 × 16–29 (28.0 × 25.0)	1.0–1.5 (1.1)	present	smooth, (1.0)	absent
	S. similis	present study	subspheroidal to ovoidal	20–26 × 19–24 (21.4 × 19.8)	1.0–1.2 (1.08)	present, 1–2 (usually only 1)	smooth, 1.2–1.8 (1.6)	inconspicuous
Isospora similisi Coelho et al., 2013	*S. similis*	Coelho et al. (2013)	subspheroidal	26–29 × 24–28 (27.5 × 25.9)	1.0–1.1 (1.1)	present, splinter-like or comma-like granules	smooth, 1.1–1.3 (1.2)	absent
	S. similis	present study	subspheroidal	20–26 × 19–24 (23.3 × 22.0)	1.0–1.1 (1.06)	present, splinter-like or comma-like granules	smooth, 1.4–1.8 (1.7)	absent

Table 1 continued

Species	Host	Reference	Sporocyst					
			Shape	Size (µm)	Shape index	Stieda body	Sub-Stieda body	Sporocyst residuum
Isospora pityli McQuistion, Capparella, 1992	*Saltator grossus* (Linnaeus)	McQuistion & Capparella (1992)	ovoidal	12–17 × 8–11 (14.7 × 9.4)	1.3–1.7 (1.57)	small, nipplelike	absent	amorphous cluster of granules, 4.0–5.0
Isospora formarum McQuistion, Capparella, 1992	*Saltator grossus* (Linnaeus)	McQuistion & Capparella (1992)	ovoidal	14–17 × 10–13 (15.7 × 11.3)	1.2–1.5 (1.4)	small, nipplelike	triangular-shaped or large conical, with irregular lower edge	large, nearly spheroid, com- posed of fine, uniform granules, (7,0)
Isospora saltatori Berto et al., 2008	*Saltator similis* d'Orbigny & Lafresnaye	Berto et al. (2008)	ovoidal	12–15 × 8–10 (13.4 × 8.9)	1.4–1.7 (1.5)	small and flattened, (0.5 × 1.5)	small, (0.7 × 1.8)	mass of granules
	S. similis	Barreto et al. (2020)[1]	ovoidal	12–20 × 7–11 (14 × 8)	1.3–2.5 (1.6)	flattened, (1.0 × 2.0)	prominent, (2.0 × 3.0)	diffuse
	S. similis	present study	ovoidal to ellipsoidal	12–15 × 7–10 (13.6 × 8.5)	1.4–1.8 (1.61)	flattened to half-moon-shaped, 0.4–0.6 × 1.2–1.6 (0.5 × 1.4)	rounded, 0.8–1.2 × 1.6–1.8 (1.0 × 1.7)	granules slightly clustered and scattered
Isospora trincaferri Berto et al., 2008	*S. similis*	Lopes et al. (2007)[2]	ovoidal	14–20 × 8–13 (16.3 × 10.8)	(1.53)	prominent	barely discernible	centered and granulated
	S. similis	Berto et al. (2008)	ovoidal	17–18 × 10–13 (17.5 × 11.5)	1.3–1.6 (1.5)	bubble-shaped, (1.7 × 2.7)	large and prominent, (2.8 × 4.2)	scattered granules
	Saltator maximus Müller	Lopes et al. (2013)	ovoidal	17–20 × 10–12 (18.4 × 11.4)	1.6–1.7 (1.62)	bubble-shaped, (1.5 × 2.5)	large and rounded, (2.5 × 3.5)	scattered or clustered granules
	S. similis	Barreto et al. (2020)[3]	ovoidal	12–23 × 9–15 (18.0 × 11.0)	1.2–2.2 (1.5)	prominent club-shaped, (2.0 × 3.0)	not reported	compact
	S. similis	present study	ovoidal to ellipsoidal	14–19 × 8–12 (15.6 × 9.7)	1.4–1.9 (1.61)	knob-like to bubble-shaped, 1.1–1.4 × 2.0–2.8 (1.3 × 2.3)	rounded to trapezoidal, 1.8–2.5 × 3.0–3.6 (2.1 × 3.4)	small granules membrane-bounded, 5.3–7.4 × 4.7–5.3 (5.9 × 5.0)

Table 1 continued

Species	Host	Reference	Sporocyst					
			Shape	Size (µm)	Shape index	Stieda body	Sub-Stieda body	Sporocyst residuum
Isospora similisi Coelho et al., 2013	*S. similis*	Coelho et al. (2013)	ellipsoidal or slightly ovoidal	15–19 × 11–13 (17.4 × 12.2)	1.2–1.7 (1.4)	knob-like, (1.4 × 2.6)	large, (2.6 × 4.5)	granules of different sizes
	S. similis	present study	ovoidal to slightly piriform	15–18 × 10–12 (16.6 × 11.0)	1.4–1.7 (1.51)	half-moon-shaped to knob-like, 0.7–1.6 × 2.1–2.7 (1.0 × 2.4)	rounded to trapezoidal, sometimes heterogeneous, 2.3–3.4 × 3.3–4.8 (2.7 × 4.1)	spherules of different sizes

[1]Originally identified as *Isospora ferri* Barreto, Vilela, Houri, Lara, Torres, Silva, Castro-Filho, Costa & Martins, 2020.

[2]Originally identified as *Isospora vanriperorum* (Levine, 1982).

[3]Originally identified as *Isospora beagai* Barreto, Vilela, Houri, Lara, Torres, Silva, Castro-Filho, Costa & Martins, 2020

are, there is a tendency for their coccidian oöcysts to be shed in greater number and size. Whereas the oöcysts in the description by Coelho et al. (2013) were from green-winged saltators recently recovered from illegal trafficking, it is concluded that they should be more stressed/immunosuppressed shedding more and larger oöcysts compared to those obtained from breeders in this study, which despite being in captivity, were better treated than those subjected to illegal trafficking.

From another point of view, despite the wide ranges of measurements of oöcysts of the three species identified in this study, these coccidians were uniform in the distribution of widths on lengths of their oöcysts, which were evaluated by linear regression obtaining values of R^2 (coefficient of determination) greater than 0.5 (Figure 5) (Berto et al. 2014). Additionally, it can be implied that the oöcysts and sporocysts of *I. trincaferri* and *I. similisi* are not differentiated by their sizes, as there is proximity of their regression lines, unlike the oöcysts and sporocysts of *I. saltatori* which are smaller, therefore their regression lines are below and to the left in the graph in Figure 5. The less sloping regression line of *I. trincaferri* also can be highlighted, which matches its ovoidal oöcysts, that is, longer than those of *I. similisi* and *I. saltatori* (Figure 5) (Berto et al. 2014).

Some characteristic features were updated and/or detailed in the reports of *I. trincaferri* and *I. similisi* in the present study, which were not mentioned in

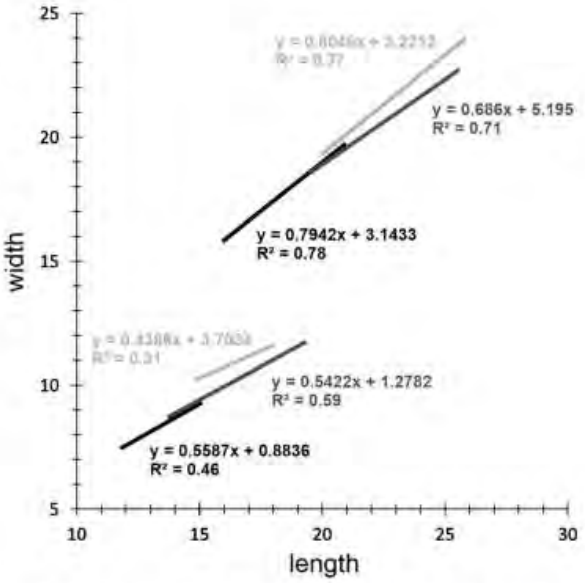

Fig. 5 Comparative linear regressions of oöcysts (above) and sporocysts (below) of *Isospora saltatori* (black), *Isospora trincaferri* (dark gray) and *Isospora similisi* (light gray) recovered from green-winged saltators *Saltator similis*.

previous studies due to observation failure or the lower number of observed oöcysts. It is noteworthy in the description of *I. trincaferri* the inclusion of an inconspicuous micropyle in some oöcysts and, in *I. similisi*, the observation of oöcysts with heterogeneous sub-Stieda bodies, that is, with different densities, resembling a compartmentalized sub-Stieda body. These inclusions are not essential for the identification

of these species, but they provide further details to their descriptions.

Isospora pityli McQuistion, Capparella, 1992 and *Isospora formarum* McQuistion, Capparella, 1992 have been described from slate-colored grosbeaks *Saltator grossus* (Linnaeus) on the east (cis-Andean) and west (trans-Andean) slopes of the Andes Mountains in Ecuador (McQuistion & Capparella, 1992). *Saltator similis* is not sympatric with *S. grossus*, making direct transmission of coccidians between these saltators unlikely, although the buff-throated saltator *S. maximus* has a wide geographic distribution in central Brazil and is sympatric with both *S. similis* and *S. grossus* (BirdLife International, 2021). This information is relevant, as *I. pityli* is similar to *I. saltatori*, just as *I. formarum* is similar to *I. similisi*, and since the recent classification of Passeriformes reclassified *Pitylus grossus* (Linnaeus) (synonymy of *S. grossus* and previously classified in the family Emberizidae) in the genus *Saltator* and the subfamily Saltatorinae in the Thraupidae family has been established (Pacheco et al., 2021), these coccidian species of *S. grossus* were approximated to the coccidians of *S. similis*. With the exception of a few characteristic features, such as the splinter-like or comma-like polar granules of *I. similisi* and the presence of sub-Stieda in *I. saltatori*, these species are morphologically and morphometrically compatible (Table 1). Thus, *I. saltatori* would become a junior synonym of *I. pityli* and *I. similisi* a junior synonym of *I. formarum*. However, due to the allopatry between *S. similis* and *S. grossus*, these small morphological differences of the oöcysts and the fact that *I. saltatori* and *I. similisi* have been identified only from *S. similis* so far, make the establishment of *I. saltatori* and *I. similisi* as synonyms of *I. pityli* and *I. formarum* early and imprudent, at least with the data currently available.

The sequencing of the three *Isospora* spp. of the present study was suitable for the identification and molecular differentiation between them and the other *Isospora* spp. previously sequenced and deposited in the GenBank for this same genic region of the COI. The phylogenetic analysis, in turn, was not conclusive in determining monophyletic groups associated with hosts, geographic regions, morphological types, etc. as already observed by other authors (Oliveira et al. 2021). In fact, this ~250bp genic region of the COI gene is easily amplified from individual oöcysts and, therefore, frequently used since the pioneering study by Dolnik et al. (2009), in addition to being indicated as the gene of the 'Barcode of Life' (Ogedengbe et al. 2011); however, the more *Isospora* spp. sequences for this genic region are deposited, the more their innappropriateness for phylogenetic conclusions is observed. Anyway, the phylogenetic analysis for these *Isospora* spp. is presented in this study to support these conclusions and, possibly, guide the future choice of other genic regions for the phylogenetic study of *Isospora* spp. of passerines.

Breeding green-winged saltators near the conservation unit of the Itatiaia National Park is a dangerous activity from an environmental point of view, as their coccidian parasites can be transmitted to *S. similis* and other susceptible *Saltator* spp. in the park. Transmission to other *Saltator* spp. it would be possible according to the report of *I. trincaferri* from *S. maximus*; furthermore, due to the widely accepted concept of host family-level specificity (Duszynski & Wilber, 1997; Berto et al. 2011), other thraupids, in addition to saltators, could also be susceptible. Additionally, as in captive birds the density of coccidians is potentially higher than in birds in the wild, subsequent transmissions of a greater number of oöcysts from captivity to the wild could increase the component population of oöcysts in certain localities and, consequently, increase the suprapopulation of coccidians in the Itatiaia National Park, which can lead to epizootics of coccidiosis depending on the species and immunity of the passerines (Bush et al. 1997; Berto & Lopes, 2020).

In this context, there are 18 *Saltator* spp., all Neotropical, of which 9 occur in Brazil (BirdLife International, 2021). In the Itatiaia National Park, three species are observed: (1) *Saltator similis*; (2) the thick-billed saltator *Saltator maxillosus* (Cabanis); and (3) the black-throated grosbeak *Saltator fuliginosus* (Daudin) (Ribenboim, 2017). Of these three species, *S. similis* is most commonly observed in the upper part of the Itatiaia National Park, but also in the lower part and in the surroundings of the park; therefore, it is the most susceptible species to approach and become infected in captivity around the Itatiaia National Park (Maia-Gouvêa et al., 2005; Ribenboim, 2017). *Saltator fuliginosus* and *S. maxillosus* are, respectively, not often seen and difficult to spot in the PNI (Maia-Gouvêa et al., 2005; Ribenboim, 2017). In this sense, *S. fuliginosus* and *S. maxillosus*, which are

more restricted in the Itatiaia National Park, have high forest dependence (BirdLife International, 2021) and are potentially susceptible to *Isospora* spp. of *S. similis*, are likely the most threatened species to this possible transmission and dispersion of coccidians of *S. similis* from captivity to the wild environment of the Itatiaia National Park.

Finally, based on the morphological and molecular features described herein, *I. saltatori*, *I. trincaferri* and *I. similisi* are reported from five captive breeding colonies of *S. similis* in the surroundings of the Itatiaia National Park, highlighting the risks ranging from transmissions to epizootics for wild saltators in the park.

Acknowledgements We are thankful to the breeders of green-winged saltators located in the surroundings of the Itatiaia National Park, for permitting and assisting with the collection of the material.

Funding This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ). CM has a scholarship from CAPES (Grant/Award Number: 001). MSO has a post-doctoral scholarship from FAPERJ (Grant/Award Number: E-26/204.228/2021). JLG-O has a scholarship from CNPq (Grant/Award Number: 142183/2020-2). BPB has fellowships from CNPq (Grant/Award Number: 303899/2019-0) and from FAPERJ (Grant/Award Number: E-26/202.797/2019).

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Collecting permits were issued by CEUA/UBM (protocol 007/2018). All applicable institutional, national and international guidelines for the care and use of animals were followed.

Data availability All data generated or analyzed during this study are included in the article.

References

Barreto, C., Vilela, D. A. R., Houri, B. F., Lara, L. B., Torres, A. C. D., Silva, A. S. G., Castro Filho, R. P. L., Costa, C. S., & Martins, N. R. S. (2020). New *Isospora* and Host Species in Brazilian Passerines. *Brazilian Journal of Poultry Science, 22*, 01–14. https://doi.org/10.1590/1806-9061-2019-1070

Berto, B. P., Balthazar, L. M. C., Flausino, W., & Lopes, C. W. G. (2008). Two new coccidian parasites of green-winged saltator (*Saltator similis*) from South America. *Acta Protozoologica*, 47, 263–267.

Berto, B. P., Flausino, W., McIntosh, D., Teixeira-Filho, W. L., & Lopes, C. W. G. (2011). Coccidia of New World passerine birds (Aves: Passeriformes): a review of *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae). *Systematic Parasitology, 80*, 159–204. https://doi.org/10.1007/s11230-011-9317-8

Berto, B. P., McIntosh, D., & Lopes, C. W. G. (2014). Studies on coccidian oöcysts (Apicomplexa: Eucoccidiorida). *Revista Brasileira de Parasitologia Veterinária, 23*, 1–15. https://doi.org/10.1590/S1984-29612014001

Berto, B. P., & Lopes, C. W. G. (2020). Coccidia of wild birds as ecological biomarkers: Some approaches on parasite-host-environment interaction. *Journal of Parasitology, 106*, 707–713. https://doi.org/10.1645/19-148

BirdLife International. (2021). IUCN Red List for birds. http://www.birdlife.org. Accessed 21 out 2021.

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology, 83, 575–583. https://doi.org/10.2307/3284227

Coelho, C. D., Berto, B. P., Neves, D. M., de Oliveira, V. M., Flausino, W., & Lopes, C. W. G. (2012). Diagnóstico e tratamento das coccidioses em trinca-ferros-verdadeiros *Saltator similis* d'Orbigny Lafresnaye, 1837 mantidos em regime de quarentena. *Brazilian Journal of Veterinary Medicine, 34*, 46–54.

Coelho, C. D., Berto, B. P., Neves, D. M., Oliveira, V. M. D., Flausino, W., & Lopes, C. W. G. (2013). Oocyst shedding by green-winged-saltator (*Saltator similis*) in the diagnostic of coccidiosis and *Isospora similisi* n. sp. (Apicomplexa: Eimeriidae). *Revista Brasileira de Parasitologia Veterinária, 22*, 64-70. https://doi.org/10.1590/S1984-29612013000100012

Destro, G. F. G., Pimentel, T. L., Sabaini, R. M., Borges, R. C., & Barreto, R. (2012). Efforts to combat wild animals trafficking in Brazil. In G. A. Lameed (Ed.), Biodiversity enrichment in a diverse world (pp. 421-436). Londres.

Dolnik, O. V., Palinauskas, V., & Bensch, S. (2009). Individual oöcysts of *Isospora* (Apicomplexa: Coccidia) parasites from avian feces: from photo to sequence. *Journal of Parasitology, 95*, 169–174. https://doi.org/10.1645/GE-1873.1

Duszynski, D. W., & Wilber, P. G. (1997). A guideline for the preparation of species descriptions in the Eimeriidae. *Journal of Parasitology, 83*, 333–336. https://doi.org/10.2307/3284470

Freitas, M. F. L., Oliveira, J. B., Cavalcanti, M. D. B., Leite, A. S., Magalhaes, V. S., Oliveira, R. A., & Evencio-Sobrino, A. (2002). Gastrointestinal parasites of captive wild birds in Pernambuco state, Brazil. *Parasitologia Latinoamericana, 57*, 50–54.

ICMBIO. (2016). Parque Nacional do Itatiaia. http://www.icmbio.gov.br/parnaitatiaia. Accessed 21 out 2021.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution, 35*, 1547–1549. https://doi.org/10.1093/molbev/msy096

Lopes, B. B., Berto, B. P., Massad, F., & Lopes, C. W. G. (2007). *Isospora vanriperorum* Levine, 1982 (Apicomplexa: Eimeriidae) in the green-winged saltator, *Saltator similis* (Passeriformes: Cardinalinae) in southeastern Brazil. *Revista Brasileira de Parasitologia Veterinária, 16*, 211–214. https://doi.org/10.1590/S1984-29612007000400006

Li, J., Xing, T., Wang, L., Tao, J., & Liu, Z. (2010). Inhibitory effect of S-nitroso-glutathione on *Eimeria tenella* oocysts was mainly limited to the early stages of sporogony. *Veterinary Parasitology, 173*, 64–69. https://doi.org/10.1016/j.vetpar.2010.06.022

Maia-Gouvêa, E. R., Gouvêa, É., & Piratelli, A. (2005). Comunidade de aves de sub-bosque em uma área de entorno do Parque Nacional do Itatiaia, Rio de Janeiro, Brasil. *Revista Brasileira de Zoologia, 22*, 859–866. https://doi.org/10.1590/S0101-81752005000400009

McQuistion, T. E., & Capparella, A. (1992). Two new coccidian parasites from the slate-colored grosbeak (*Pitylus grossus*) of South America. *Journal of Parasitology, 78*, 805–807. https://doi.org/10.2307/3283308

Norton, C. C., & Joyner, L. P. (1981). *Eimeria acervulina* and *E. mivati*: oocysts, life-cycle and ability to develop in the chicken embryo. Parasitology, 83, 269–279. https://doi.org/10.1017/S0031182000085280

Ogedengbe, J.D., Hanner, R.H., & Barta, J.R. (2011). DNA barcoding identifies *Eimeria* species and contributes to the phylogenetics of coccidian parasites (Eimeriorina, Apicomplexa, Alveolata). *International Journal for Parasitology, 41*, 843–850. https://doi.org/10.1016/j.ijpara.2011.03.007

Oliveira, P. S. D., Ferreira, M. A., Silva, L. M. D., Rodrigues, M. B., Cardozo, S. V., & Berto, B. P. (2017). Diversity and distribution of coccidia of wild birds in an Atlantic forest fragment area in southeastern Brazil. *Revista Brasileira de Parasitologia Veterinária, 26*, 457–464. https://doi.org/10.1590/S1984-29612017063

Oliveira, M. S., Mello, E. R., Cardozo, S. V., Oliveira, A. A., Lima, V. M., Ferreira, I., Berto, B. P. (2021) Distribution, redescription and molecular identification of Isospora striata McQuistion et. al 1997 (Eimeriidae) from woodcreepers (Dendrocolaptidae) in South America. Parasitology Research, 120, 2585–2593. https://doi.org/10.1007/s00436-021-07140-9

Pacheco, J. F., Silveira, L. F., Aleixo, A., Agne, C. E., Bencke, G. A., Bravo, G. A., Brito, G. R. R., Cohn-Haft, M., Maurício, G. N., Naka, L. N., Olmos, F., Posso, S. R., Lees, A. C., Figueiredo, L. F. A., Carrano, E., Guedes, E. C., Cesari, E., Franz, I., Schunck, F., Piacentini, V. Q. (2021) Annotated checklist of the birds of Brazil by the Brazilian Ornithological Records Committee - second edition. *Ornithology Research, 29*, 94–105. https://doi.org/10.1007/s43388-021-00058-x

Sick, H. (1997). Ornitologia Brasileira. Rio de Janeiro: Nova Fronteira, 862 pp.

Ribenboim, L. C. D. (2017). Aves do Parque Nacional do Itatiaia. Taubaté: Dica de Turismo Publicações, 67 pp.

Yang, R., Brice, B., Elliot, A., & Ryan, U. (2015). *Isospora serinuse* n. sp. (Apicomplexa: Eimeriidae) from a domestic canary (*Serinus canaria forma domestica*) (Passeriformes: Fringillidae) in Western Australia. *Experimental Parasitology, 159*, 59–66. https://doi.org/10.1016/j.exppara.2015.08.020

Parasitology Research
https://doi.org/10.1007/s00436-022-07464-0

PROTOZOOLOGY - SHORT COMMUNICATION

Observations on an *Eimeria* sp. (Apicomplexa) from the green kingfisher *Chloroceryle americana* (Gmelin, 1788) (Coraciiformes) in Southeastern Brazil: an example of how the ecological aspects of the host can be essential for the identification of its coccidians

Lucas de Assis Silva Andrade[1] · Jhon Lennon Genovez-Oliveira[1] · Mariana de Souza Oliveira[1] · Ericson Ramos de Mello[2] · Sergian Vianna Cardozo[3] · Águida Aparecida de Oliveira[4] · Viviane Moreira de Lima[5] · Ildemar Ferreira[5] · Bruno Pereira Berto[5]

Received: 14 December 2021 / Accepted: 8 February 2022

Abstract
Parasitism in kingfishers is very little reported and predominantly related to hemoparasites, helminths, and ectoparasites. The present study provided a morphological and genotypic study of an *Eimeria* sp. recovered from a green kingfisher *Chloroceryle americana* (Gmelin, 1788) captured in the Marambaia Island, on the coast of the state of Rio de Janeiro, Southeastern Brazil. The coccidial density, some morphological aspects of its oocysts, the molecular results, and, mainly, the ecological niche of *C. americana* in the mangrove of the Marambaia Island suggest that this coccidian species is a pseudoparasite.

Keywords Pseudoparasitism · Fish coccidia · Oocysts · Morphology · Sequencing · Marambaia Island

Introduction

Kingfishers are coraciiform birds that belong to the Alcedinidae family, which comprises 120 species worldwide (IUCN 2021). They have a wide geographic distribution, with most species occurring in tropical and subtropical zones, close to aquatic environments (Sick 1997). In Brazil, there are five species, four of the genus *Chloroceryle* Kaup, 1848 plus the ringed kingfisher *Megaceryle torquata* (Linnaeus, 1766) (Pacheco et al. 2021).

The green kingfisher *Chloroceryle americana* (Gmelin, 1788) is the most common alcedinid bird in Brazil, where it lives along rivers, lakes, coastlines, mangroves, and river mouths. This species feeds mainly on fish from 3 to 5.5 cm; however, it is a very generalist species and is also observed feeding on small vertebrates and invertebrates (Sick 1997; WikiAves 2021).

Parasitism in *C. americana* is very little reported and restricted to hemoparasites, helminths, and ectoparasites (Roda and Farias, 1999; López-Jiménez et al. 2018; Carvalho et al. 2021), although there is a report of an *Eimeria* sp. from green kingfishers in the Municipality of Rio Branco in the State of Acre in Northern Brazil (Almeida-Brito et al., 2017). In this context, the present study aims to report oocysts from an *Eimeria* sp. recovered from fecal samples of a *C. americana* captured in the mangrove of Marambaia Island, on the Brazilian coast, associating this finding with a possible pseudoparasitism. Additionally, the present study provided a genotypic identification, in addition

Section Editor: Berit Bangoura.

✉ Bruno Pereira Berto
bertobp@ufrrj.br

[1] Programa de Pós-Graduação Em Biologia Animal, Instituto de Ciências Biológicas E da Saúde (ICBS), Universidade Federal Rural Do Rio de Janeiro (UFRRJ), BR-465 km 7, Seropédica, RJ 23897-000, Brazil

[2] Programa de Pós-Graduação Em Ciências Veterinárias, Instituto de Veterinária (IV), UFRRJ, BR-465 km 7, Seropédica, RJ 23897-000, Brazil

[3] Programa de Pós-Graduação Em Biomedicina Translacional, Universidade Do Grande Rio, Rua Professor José de Souza Herdy 1160, Duque de Caxias, RJ 25071-202, Brazil

[4] Departamento de Microbiologia E Imunologia Veterinária, UFRRJ, BR-465 km 7, Seropédica, IVRJ 23897-000, Brazil

[5] Departamento de Biologia Animal, ICBS, UFRRJ, BR-465 km 7, Seropédica, RJ 23897-000, Brazil

Published online: 14 February 2022

to morphological study for this *Eimeria* sp., via sequencing of the mitochondrial cytochrome *c* oxidase subunit 1 (COI) gene.

Materials and methods

Sample collection

Two expeditions in June 2019 and November 2021 were conducted in the mangrove of Marambaia Island (23°3′52.90″S, 43°59′22.80″W), on the coast of the state of Rio de Janeiro, Southeastern Brazil, where five *C. americana* were captured with mist nets (two in 2019 and three in 2021). The kingfishers were kept in individual boxes and fecal droplets collected immediately after defecation. After identification of the species, the birds were photographed and released and fecal droplets were placed in centrifuge tubes containing a potassium dichromate 2.5% ($K_2Cr_2O_7$) solution at 1:6 (v/v).

Morphological analysis

Samples were transported to the Laboratório de Biologia de Coccídios, Universidade Federal Rural do Rio de Janeiro (UFRRJ), incubated at 23–28 °C, and regularly oxygenated by shaking of the centrifuge tubes for 7 days. Oocysts were isolated by flotation in Sheather's sugar solution (Specific gravity: 1.20) and examined microscopically using the technique described by Duszynski and Wilber (1997) and Berto et al. (2014). Morphological observations, line drawings, photomicrographs, and measurements were made using an Olympus BX binocular microscope (Olympus Optical, Tokyo, Japan) coupled to a digital camera Eurekam 5.0 (BEL Photonics, Monza, Italy). Line drawings were edited using two software applications from CorelDRAW® (Corel Draw Graphics Suite, Version 11.0, Corel Corporation, Canada), i.e., Corel DRAW and Corel PHOTO-PAINT. All measurements are in micrometers and are given as the range followed by the mean in parentheses.

Molecular analysis

Twelve oocysts of the same morphotype under light microscopy, which were recovered from a single fecal droplet, were isolated, resuspended in PBS, and washed by centrifuging until the supernatant became clear (Dolnik et al. 2009). DNA was extracted from the oocysts using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, São Paulo, Brazil) according to the manufacturer's instructions. In order to fully lyse the oocysts, four freeze–thaw cycles were applied prior to the DNA extraction. The PCR amplification for the COI gene was carried out as previously described by Genovez-Oliveira et al. (2020). The primers: JAVF (5′-CTGAATTTG GTTCAGGTGTTGGT-3′) and JAVR (5′-TACACCAGT AGTACCTCCAAGGG-3′) produced a PCR product size of ~ 651pb. For amplification, a 25-µl PCR reaction was prepared using 3µL of genomic DNA (< 1 µg), 12.5µL of GoTaq® G2 Hot Start Colorless Master Mix (Promega Labs) (1X), 0.25 µL of each Primer (0.2 µM), and 9µL of Nuclease Free Water. PCR was conducted using the following cycling conditions: 1 cycle of 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 53 °C for 45 s, and 72 °C for 50 s and a final extension of 72 °C for 5 min. The amplicons were purified using the Qiagen MinElute PCR Purification (Qiagen, São Paulo, Brazil). The PCR product was sequenced using the PCR forward and reverse primers by Ludwig Biotechnology, where an ABI-Prism 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA) was used for Sanger sequencing. The results of the sequencing reactions were analyzed and edited using the program Chromas 2.6. Sequences were compared with other coccidian parasites available on the GenBank database using the Basic Local Alignment Search Tool (BLAST). Phylogenetic trees were constructed for coccidian species at the COI sequences aligned with additional isolates from GenBank. *Toxoplasma gondii* (Nicolle & Manceaux, 1908) was used as the outgroup. Distance analyses and phylogenies were conducted using MEGA X (Kumar et al. 2018). Briefly, Sanger sequencing chromatogram files were imported into MEGA X and the nucleotide sequences of each genic region were curated, analyzed, and aligned with reference sequences from GenBank using Clustal W (http://www.clustalw.genome.jp). Maximum likelihood (ML) and neighbor-joining (NJ) trees were constructed and the distances were computed using the Tamura-Nei method based on model selection using ModelTest in MEGA X. Bootstrap analyses were conducted using 1000 replicates to assess the reliability of inferred tree topologies.

Results and discussion

Of the five green kingfishers examined, only one of them captured in June 2019 was passing coccidian oocysts in their feces. More precisely, of the five fecal droplets collected from this kingfisher, only two had 8 and 12 oocysts. These oocysts (Fig. 1A–E) are subspherical, 14.7 (14–15) × 13.8 (13–14), with length/width (L/W) ratio of 1.07 (1.0–1.1). Oocyst wall bi-layered and smooth, about 1.0 thick. Micropyle, oocyst residuum is absent, but 1–2 small polar granules are present. Sporocysts irregularly ovoidal, 9.6 (9–10) × 5.6 (5–6) µm, with L/W ratio of 1.72 (1.5–1.9). Stieda body thin and flattened, 0.5 high × 1.0 wide. Substieda body rounded, 1.0 high × 1.5 wide. Parastieda body absent. Sporocyst residuum present, consisting of numerous granules that appear to be membrane-bounded. Sporozoites with anterior and posterior refractile bodies.

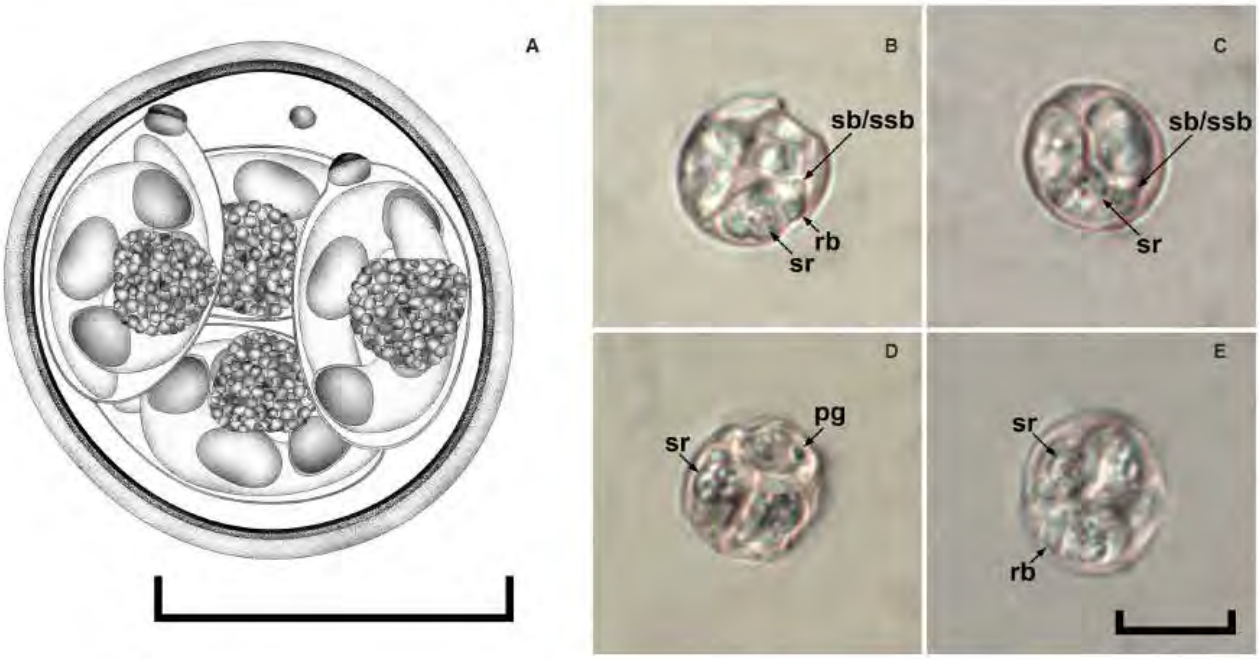

Fig. 1 Composite line drawing (**A**) and photomicrographs (**B–E**) of sporulated oocysts of an *Eimeria* sp. recovered from a green kingfisher *Chloroceryle americana*. Note the polar granule (pg); Stieda and sub-Stieda bodies (sb/ssb); sporocyst residuum (sr); and refractile body (prb). Scale bar: 10 µm

Photomicrographs, line drawing, and oocysts in 2.5% $K_2Cr_2O_7$ solution (Williams et al. 2010) are deposited and available (http://r1.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under repository number 119/2021. Photovouchers of the *C. americana* specimen are deposited in the same collection.

The only descriptive reports of *Eimeria* spp. from alcedinid birds were by Varghese (1977) who described *Eimeria galateai* Varghese, 1977 from common paradise-kingfishers *Tanysiptera galatea* Gray, 1859 and *Eimeria duncani* Varghese, 1977 from sacred kingfishers *Todiramphus sanctus* (Vigors & Horsfeld, 1827) in Papua New Guinea. *Eimeria galateai* is similar in size to *Eimeria* sp. of this study; however, it is different for its elongate ovoidal oocysts with protrusions on the ends. *Eimeria duncani* has ovoidal oocysts truncated at the narrower end and is larger than the oocysts of this study. In Varghese (1977), kingfishers were kept in individual, coccidia-free cages, and fed on larval and adult insects, reducing the risk of contamination and/or pseudoparasitism by predation on parasitized prey. Furthermore, fresh fecal samples were obtained with hundreds of unsporulated oocysts that ensured the host-parasite relationship between kingfishers and *Eimeria* spp.

Alternately, some aspects of the finding of this *Eimeria* sp. from *C. americana* on Marambaia Island raise doubts whether this is a true parasitic relationship. First, the research group of Prof. Bruno Pereira Berto has been researching coccidia of birds on Marambaia Island since 2007 (Lopes et al. 2013), where more than a hundred green kingfishers have been captured since then, all of which, to date, were coccidia-negative. The island has three predominant biomes: Atlantic Forest, Restinga, and mangrove; however, the vast majority of expeditions conducted by the research group on the island were carried out in areas of Atlantic Forest, with only the June 2019 and November 2021 expeditions being carried out in the mangrove, where the abundance and diversity of prey for *C. americana* is quite different (Sick 1997). In this context, from an ecological point of view, it is very reasonable that these oocysts are, in fact, parasites of some prey of *C. americana* in the mangrove of Marambaia Island, mainly fish, but also other animals that may be ingested, digested, and eliminated by green kingfishers. Secondarily, the low number of oocysts observed in less than half of the collected fecal droplets and oocysts with shriveled walls (even in short time in saturated solution) suggests that these oocysts are possibly not parasites of this green kingfisher captured in 2019. This hypothesis of pseudoparasitism is similar to that addressed by Trefancová et al. (2019) for isosporan coccidia recovered from rodents that would be their pseudoparasites and true parasites of passerines.

In order to confirm this hypothesis in the present study, in the expedition in the mangrove of the Marambaia Island

Fig. 2 Maximum likelihood tree estimated from the COI sequences. Numbers at nodes represent bootstrap support (1000 replicates; only values> 50% shown) for neighbor-joining and maximum likelihood, respectively. The scale bar represents the number of nucleotide substitutions per site

in 2021, some poeciliid fish were caught in the mangrove streams close to the localities where the green kingfishers were captured with mist nets, but these fish were coccidia-negative. It is noteworthy that, if this pseudoparasitism hypothesis is correct, the identification of the true host of this *Eimeria* sp. is quite difficult, as the feeding habits of green kingfishers can be quite generalist, especially in the mangrove of the Marambaia Island, which offers a great diversity of fish, amphibians, reptiles, crustaceans, and other animals that are potential prey (Sick 1997; Luz et al. 2008; Santos 2009; WikiAves 2021). Another option to determine if the kingfishers are the true hosts of this *Eimeria* sp. is to euthanize the kingfishers and examine the intestinal tract histologically for endogenous stages of the coccidian; however, in Brazil, the native birds are protected by law and supervised by IBAMA (Brazilian Institute of the Environment and Natural Renewable Resources). In any case, the morphology of the oocysts observed from the green kingfisher in this study is typical of *Eimeria* spp. of fish, both in relation to size, since fish coccidia tend to have small oocysts, as well as the morphology of the oocyst wall, which tends to be more delicate and shrivel easily (Molnár and Fernando 1974; Dyková and Lom 1981; Molnár 2000; Couso-Pérez et al. 2019). This delicate wall is also typical of *Goussia* spp., which are predominantly reported from fish (Molnár 2000); however, the oocysts in the present study had constant four sporocysts per oocyst, with Stieda and sub-Stieda bodies, which are inherent characteristics of the *Eimeria* genus.

Molecular identification by the COI gene (GenBank accession number: OL773690) reinforces the hypothesis that this *Eimeria* sp. is distant from *Eimeria* spp. of birds, since the highest genotypic similarities were only 93% with *Eimeria* spp. of rodents, primates, canids, bats, psittaciforms, and galliforms. Thus, the phylogenetic analysis placed this *Eimeria* sp. of *C. americana* separated from a clade with *Eimeria* spp. of mammals and birds (Fig. 2). It is assumed that *Eimeria* sp. of this study was not molecularly close to *Eimeria* spp. of fish due to not having deposits of fish coccidia for COI gene in GenBank. In fact, *Eimeria* spp. from fish deposited in GenBank were predominantly sequenced for ribosomal genes (Molnár et al. 2012) and, therefore, in the present study, sequencing for genic regions of the 18S and 28S ribosomal genes was also intended, but the amplifications for these genes were not successful. Anyway, in the study with ribosomal genes by Molnár et al. (2012), it was observed that *Eimeria* spp. of fish are related to *Eimeria* spp. of mammals and birds, similarly to what was observed in the phylogeny of the present study; that is, the phylograms have similar topology in these studies even using different genes, with the fish coccidia clustering adjacent to the *Eimeria* spp. of mammals and birds (Fig. 2).

In conclusion, the present study identifies strong ecological, morphological, and molecular evidence that suggest a pseudoparasism scenario of this *Eimeria* sp. from *C. americana* in the mangrove of Ilha da Marambaia, which true host is a fish or other prey item ingested by the kingfisher and then shed in its feces.

Acknowledgements We are thankful to staff at the Brazilian Navy, especially to the command of CADIM (Centro de Avaliação da Ilha da Marambaia), that allowed us to access and use some facilities during the expeditions.

Funding This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação

de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ). LASA has a scholarship from CAPES (Grant/Award Number: 001). JLG-O has a scholarship from CNPq (Grant/Award Number: 142183/2020–2). MSO has a post-doctoral scholarship from FAPERJ (Grant/Award Number: E-26/204.228/2021). BPB has fellowships from CNPq (Grant/Award Number: 303899/2019–0) and from FAPERJ (Grant/Award Number: E-26/202.797/2019).

Declarations

Ethical approval Field-collecting permits were issued by SISBIO/ICMBio (licenses 54951; 70132) and CEUA (protocols UFRRJ-IV-6606250616; UNIGRANRIO-021/2019). All applicable institutional, national, and international guidelines for the care and use of animals were followed.

Conflict of interest The authors declare no competing interests.

References

Almeida-Brito AS, Guilherme E, Araújo-Santos FG, Mesquita RP, Gomes FA (2017) Endoparasites of wild birds from campus area and zoobotanical park, at the federal university of Acre, Rio Branco-Acre. Arq Ciê Vet Zool UNIPAR 20:117–122

Berto BP, McIntosh D, Lopes CWG (2014) Studies on coccidian oocysts (Apicomplexa: Eucoccidiorida). Rev Bras Parasitol Vet 23:1–15

Carvalho AM, Ferreira FC, Araújo AC, Hirano LQL, Paludo GR, Braga ÉM (2021) Molecular detection of *Leucocytozoon* in red-legged seriemas (*Cariama cristata*), a non-migratory bird species in the Brazilian Cerrado. Vet Parasitol Reg Stud Reports 100652

Couso-Pérez S, Ares-Mazás E, Gómez-Couso H (2019) First molecular data on *Eimeria truttae* from brown trout (*Salmo trutta*). Parasitol Res 118:2121–2127

Dyková I, Lom J (1981) Fish coccidia: critical notes on life cycles, classification and pathogenicity. J Fish Dis 4:487–505

Dolnik OV, Palinauskas V, Bensch S (2009) Individual oocysts of *Isospora* (Apicomplexa: Coccidia) parasites from avian feces: from photo to sequence. J Parasitol 95:169–174

Duszynski D, Wilber P (1997) A guideline for the preparation of species descriptions in the Eimeridae. J Parasitol 83:333–336

Genovez-Oliveira JL, Oliveira MS, Thode-Filho S, Cardozo SV, Oliveira ÁA, Lima VM, Ferreira I, Berto BP (2020) Morphological and molecular identification of *Isospora massardi* Lopes, Berto, Luz, Galvão, Ferreira & Lopes, 2014 (Chromista: Miozoa: Eimeriidae) from thrushes *Turdus* spp. (Passeriformes: Turdidae) in South America. Parasitol Int 75:102040

IUCN (2021) The IUCN Red List of Threatened Species. Available from: <http://www.iucnredlist.org>. Accessed 03 Dec 2021

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549

Lopes BB, Berto BP, Ferreira I, Luz HR, Lopes CWG (2013) Coccidial distribution from passerines in an area of Atlantic Forest in Marambaia Island, Rio de Janeiro, Brazil. Coccidia 1:10–16

López-Jiménez A, De LeÓn GPP, García-Varela M (2018) Molecular data reveal high diversity of Uvulifer (Trematoda: Diplostomidae) in Middle America, with the description of a new species. J Helminthol 92:725–739

Luz HR, Costa LM, Prata ÁF, Moraes D, Ferreira I, Esbérard CEL (2008) Chloroceryle americana (Gmelin, 1788)(Aves: Coraciiformes) predando lagarto Gymnophtalmidae. Lundiana 9:77–77

Molnár K, Fernando CH (1974) Some new Eimeria (Protozoa, Coccidia) from freshwater fishes in Ontario. Canada Can J Zool 52:413–419

Molnár K (2000) Two new coccidia, a *Goussia* and an *Eimeria* spp. from the gut of Kessler's goby (*Gobius kessleri* Günther) in the River Danube. Acta Protozool 39:323–329

Molnár K, Ostoros G, Dunams-Morel D, Rosenthal BM (2012) Eimeria that infect fish are diverse and are related to, but distinct from, those that infect terrestrial vertebrates. Infect Genet Evol 12:1810–1815

Pacheco JF, Silveira LF, Aleixo A, Agne CE, Bencke GA, Bravo GA, Brito GRR, Cohn-Haft M, Maurício GN, Naka LN, Olmos F, Posso SR, Lees AC, Figueiredo LFA, Carrano E, Guedes EC, Cesari E, Franz I, Schunck F, Piacentini VQ 2021 Annotated checklist of the birds of Brazil by the Brazilian Ornithological Records Committee - second edition. Ornithol Res 29:94–105

Roda SA, Farias ÂMID (1999) Wild birds infested by Phthiraptera (Insecta) in Pernambuco North Zona da Mata. Rev Bras Zool 16:871–878

Santos EM (2009) Notas sobre predação de anuros em uma poça temporária no nordeste do Brasil. Bol Mus Biol Mello Leitão 25:77–82

Sick H (1997) Ornitologia Brasileira. Nova Fronteira, Rio de Janeiro, p 862

Trefancová A, Mácová A, Kvičerová J (2019) Isosporan oocysts in the faeces of bank voles (Myodes glareolus; Arvicolinae, Rodentia): real parasites, or pseudoparasites? Protist 170:104–120

Varghese T (1977) Eimeria galateai sp. n. from the paradise kingfisher, and Eimeria duncani sp. n. from the sacred kingfisher in Papua New Guinea. J Protozool 24:209–210

WikiAves (2021) Wiki Aves - A Enciclopédia das Aves do Brasil. Available from: <https://www.wikiaves.com.br>. Accessed 03 Dec 2021

Williams RB, Thebo P, Marshall RN, Marshall JA (2010) Coccidian oöcysts as type–specimens: long–term storage in aqueous potassium dichromate solution preserves DNA. Syst Parasitol 76:69–76

Current Research in Parasitology & Vector-Borne Diseases 2 (2022) 100079

Contents lists available at ScienceDirect

Current Research in Parasitology & Vector-Borne Diseases

journal homepage: www.editorialmanager.com/crpvbd/default.aspx

Isospora basileuterusi n. sp. (Apicomplexa: Eimeriidae) from the golden-crowned warbler *Basileuterus culicivorus* (Deppe) (Passeriformes: Parulidae) in South America

Ericson R. Mello [a], Mariana S. Oliveira [b], Lucas de Assis S. Andrade [b], Sergian V. Cardozo [c], Águida A. Oliveira [d], Viviane M. Lima [e], Bruno P. Berto [e,*]

[a] Programa de Pós-graduação em Ciências Veterinárias, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil
[b] Programa de Pós-graduação em Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil
[c] Programa de Pós-Graduação em Biomedicina Translacional, Universidade do Grande Rio, Duque de Caxias, Rio de Janeiro, Brazil
[d] Departamento de Microbiologia e Imunologia Veterinária, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil
[e] Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro, Brazil

ARTICLE INFO

Keywords:
Coccidia
Isospora
Oöcyst morphology
Sequencing *cox*1
New species
Neotropical birds
Parulidae
Parque Nacional do Itatiaia

ABSTRACT

Isospora basileuterusi Mello & Berto n. sp. is described based on material from the golden-crowned warbler *Basileuterus culicivorus* (Deppe) captured in the Itatiaia National Park (Parque Nacional do Itatiaia), a conservation unit in south-eastern Brazil. Oöcysts of the new species are ellipsoidal to ovoidal, measuring on average 25.2 × 21.1 µm, with a smooth, bi-layered wall, *c.*1.6 µm thick. Micropyle and oöcyst residuum are both absent, but one to three polar granules are present. Sporocysts are ellipsoidal to lemon-shaped, measuring on average 15.3 × 9.5 µm, with a knob-like Stieda body and a trapezoidal sub-Stieda body. Sporocyst residuum is present, usually as a body of membrane-bound granules. Sporozoites are vermiform, with refractile bodies. Four of the 19 warblers captured (21%) were infected with the new species. Molecular analysis of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) gene revealed a similarity of 99.5% between the new species and *Isospora serinuse* Yang, Brice, Elliot & Ryan, 2015 from island canaries *Serinus canaria* (L.) in Western Australia. The oöcysts of *I. basileuterusi* n. sp. can be distinguished from the four other *Isospora* spp. recorded in hosts of the Parulidae, and from the molecularly most closely related species, by the typical ellipsoidal to lemon-shaped sporocysts, with small sub-Stieda body and a membrane-bound sporocyst residuum. Therefore, based on the morphological and molecular features, *I. basileuterusi* n. sp. is the fifth species described in a host of the family Parulidae and the first molecularly characterized *via* sequencing the *cox*1 gene.

1. Introduction

Brazil is the second country in the Neotropical region with the highest number of bird species, with about 1971 species listed by the Brazilian Ornithological Records Committee (Pacheco et al., 2021), which corresponds to half of the of the diversity of the Neotropical avifauna (Dias, 1992). In relation to research on Neotropical birds, the study of their parasites has been highlighted for their association with ecology, biology and species conservation. Among their parasites, coccidian protozoans are important as a cause of morbidity and mortality, especially in captive birds or impacted environments, thus acting as ecological biomarkers (Berto & Lopes, 2020).

The golden-crowned warbler *Basileuterus culicivorus* (Deppe) is a passerine bird of the family Parulidae with a wide distribution in the Neotropical region (Sick, 1997; Pacheco et al., 2021). It has insectivorous eating habits and occupies the middle stratum of the ombrophilous forests (Marini & Cavalcanti, 1993; Lima & Manhães, 2009). The present study provides a description and molecular characterization of a new

* Corresponding author. Departamento de Biologia Animal, Instituto de Ciências Biológicas e da Saúde, Universidade Federal Rural do Rio de Janeiro, BR-465 km 7, Seropédica, RJ, 23897-000, Brazil.
E-mail address: bertobp@ufrrj.br (B.P. Berto).

https://doi.org/10.1016/j.crpvbd.2022.100079
Received 29 December 2021; Received in revised form 19 January 2022; Accepted 24 January 2022

species of *Isospora* from golden-crowned warblers *B. culicivorus* captured in the Itatiaia National Park (Parque Nacional do Itatiaia), a conservation unit in south-eastern Brazil.

2. Materials and methods

2.1. Sample collection

A total of 9 expeditions were conducted between 2014 and 2019 in the Itatiaia National Park, a protected area with a high degree of vulnerability, located in the Serra da Mantiqueira on the border of the States of Rio de Janeiro, Minas Gerais and São Paulo (ICMBIO, 2021), in August (22°26′19″S, 44°37′23″W) and November (22°26′57″S, 44°36′25″W) 2014; March (22°27′38″S, 44°35′34″W) 2015; March (22°19′46″S, 44°32′11″W) and October (22°27′38″S, 44°35′34″W) 2016; July (22°26′15″S, 44°18′33″W) and November (22°26′57″S, 44°36′25″W) 2017; August (22°26′57″S, 44°36′25″W) 2018; March (22°26′17″S; 44°37′33″W) 2019. A total of 19 *B. culicivorus* were captured with mist nets. The birds were kept in individual boxes and faeces collected immediately after defecation. After identification to the species level, the bird was photographed and released, and stool samples were placed in centrifuge tubes containing 2.5% potassium dichromate ($K_2Cr_2O_7$) solution at 1:6 (v/v).

2.2. Morphological analyses

Samples were examined at the Laboratório de Biologia de Coccídios, Universidade Federal Rural do Rio de Janeiro (UFRRJ). All samples were incubated at room temperature (25 °C) for 10 days or until c.70% of the oöcysts were sporulated. Oöcysts were isolated by flotation in Sheather's sugar saturated solution (specific gravity: 1.20) and examined microscopically using the technique described by Duszynski & Wilber (1997) and Berto et al. (2014a). Morphological observations, line drawings, photomicrographs and measurements were made using an Olympus BX binocular microscope (Olympus Optical, Tokyo, Japan) equipped with a digital camera Eurekam 5.0 (BEL Photonics, Monza, Italy). Line drawings were edited using two software applications (Corel DRAW and Corel PHOTO-PAINT) from CorelDRAW® (Corel Draw Graphics Suite, Version, 2020; Corel Corporation, Canada). All measurements are in micrometres and are given as the range followed by the mean in parentheses.

2.3. Molecular data generation

An individual oöcyst was isolated from serial dilutions of the oöcysts in drops on a microscope slide using a sterile micropipette. This isolated oöcyst was resuspended in PBS and washed by centrifuging until the supernatant became clear (Dolnik et al., 2009). DNA was extracted from the oöcyst using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, São Paulo, Brazil) according to the manufacturer's instructions. Four freeze-thaw cycles were applied prior to DNA extraction in order to achieve complete lysis of the oöcysts. PCR amplification of a partial fragment of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox*1) gene (c.250 bp) was carried out using nested PCR, as previously described by Dolnik et al. (2009) and Yang et al. (2015). The external primers COIbF1 (5′-GWT CAT TAG TAT GGG CAC ATC A-3′) and COIbR1 (5′-CCA AGA GAT AAT ACR AAR TGG AA-3′), produced an amplicon of c.302 bp in size and the internal primes COIbF2 (5′-GGG CAC ATC ATA TGA C-3′) and COIbR2 (5′-ATA GTA TGT ATC ATG TAR WGC AA-3′) produced an amplicon of 257 bp in size. The PCR reaction contained 12.5 μl of GoTaq® G2 Hot Start Colorless Master Mix (Promega Labs, São Paulo, Brazil) (1 ×), 0.25 μl of each primer (0.2 μM), 9 μl of nuclease-free water and 3 μl of DNA (for the primary reaction) or 3 μl primary PCR product (for the secondary reaction). Both primary and secondary PCR amplifications were conducted using the same cycling conditions: 1 cycle at 94 °C for 5 min, followed by 35 cycles (94 °C for 30 s, 47 °C for 45 s, and 72 °C for 1 min) and a final extension step at 72 °C for 5 min. The amplicons from the second round PCRs were purified using the Qiagen MinElute PCR Purification (Qiagen, São Paulo, Brazil).

2.4. DNA sequence analyses

All PCR amplicons were sequenced using the PCR forward and reverse primers by Ludwig Biotechnology, where an ABI-Prism 3500 Genetic Analyzer (Applied Biosystems, Foster City, California) was used for Sanger sequencing. The results of the sequencing reactions were analyzed and edited using the program Chromas 2.6. The newly generated sequence was compared to those for *Isospora* spp. and other coccidian parasites available in the GenBank database using the Basic Local Alignment Search Tool (BLAST). The phylogenetic tree was constructed using the newly generated *cox*1 sequence aligned with sequences for 18 species of *Isospora* available on GenBank. Distance analyses and phylogenies were conducted using MEGA X (Kumar et al., 2018). Briefly, Sanger sequencing chromatogram files were imported into MEGA X and the nucleotide sequences were curated, analyzed, and aligned with reference sequences from GenBank using Clustal W (http://www.clustalw.genome.jp). Maximum likelihood (ML) and Neighbor-Joining (NJ) trees were constructed, and the distances computed using the Tamura-Nei method based on model selection using ModelTest in MEGA X. Bootstrap analyses were conducted using 1000 replicates to assess the reliability of inferred tree topologies.

3. Results

Nineteen *B. culicivorus* were examined and four (21%) were positive for coccidian oöcysts of a morphotype not reported in the scientific literature. These positive warblers were captured in November 2014 and August 2018 on a trail named "Trilha das Borboletas" (Trail of the Butterflies) (22°26′57″S, 44°36′25″W), and in March 2019 at the starting point of the "Travessia Ruy Braga" (Ruy Braga Crossing) (22°26′17″S; 44°37′33″W) in the Itatiaia National Park. This material is described below.

3.1. *Isospora basileuterusi* Mello & Berto n. sp.

3.1.1. Taxonomic summary

Type-host: Basileuterus culicivorus (Deppe) (Passeriformes: Parulidae), warbler.

Type-locality: Parque Nacional do Itatiaia (22°26′57″S, 44°36′25″W), Brazil.

Type-material: Photosyntypes, line drawing and oöcysts in 2.5% $K_2Cr_2O_7$ solution (Williams et al., 2010) are deposited and available (http://r1.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under the repository number P-124/2021. Photographs of the type-host specimen (symbiotype) are deposited in the same collection.

Site in host: Unknown.

Prevalence: 21% (4 out of 19 birds examined).

Representative DNA sequence: One representative *cox*1 sequence was deposited in the GenBank database under the accession number OM025014.

ZooBank registration: To comply with the regulations set out in Article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN, 2012) details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:DC2F625C–B798-46DE-84F6–F6883A90E4A5. The LSID for the new name *Isospora basileuterusi* Mello & Berto n. sp. is urn:lsid:zoobank.org:act:26689BA2-AAC9-4E41-B90E-E40C5934137B.

Etymology: The specific epithet is derived from the genus name of the type-host.

Fig. 1 Composite line drawing of the sporulated oöcyst of *Isospora basileuterusi* from the golden-crowned warbler *Basileuterus culicivorus*. *Scale-bar*: 10 μm.

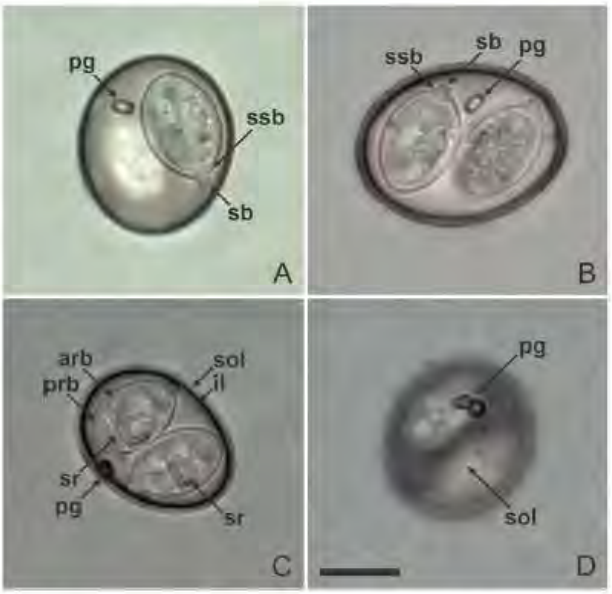

Fig. 2 Photomicrographs of sporulated oöcysts of *Isospora basileuterusi* from the golden-crowned warbler *Basileuterus culicivorus*. *Abbreviations*: inner (il) and smooth outer (sol) layers of the oöcyst wall; polar granule (pg); Stieda (sb) and sub-Stieda bodies (ssb); sporocyst residuum (sr); posterior (prb) and anterior (arb) refractile bodies. All to same scale. *Scale-bar*: 10 μm.

3.1.2. Description

[Based on 25 oöcysts and 25 sporocysts; Figs. 1 and 2.] Oöcysts ellipsoidal to ovoidal, 22–28 × 17–23 (25.2 × 21.1); L/W ratio 1.1–1.3 (1.20). Wall bi-layered, 1.5–1.9 (1.6) thick, outer layer smooth. Micropyle and oöcyst residuum both absent, but one to three (usually one) polar granules present, 2.4–3.0 × 1.7–2.4 (2.7 × 2.0). Sporocysts 2, ellipsoidal to lemon-shaped, 14–17 × 8–11 (15.3 × 9.5); L/W ratio 1.4–1.8 (1.61). Stieda body present, knob-like, 0.9–1.1 × 1.7–2.1 (1.0 × 1.8); sub-Stieda present, trapezoidal, 1.1–1.7 × 2.5–2.9 (1.4 × 2.7); para-Stieda body absent; sporocyst residuum present, usually a distinctly ovoidal to ellipsoidal body consisting of numerous small granules that appear to be membrane-bound, 4.3–5.2 × 3.5–4.3 (3.9 × 4.8). Sporozoites 4, vermiform, with anterior and posterior refractile bodies and indiscernible nucleus.

3.1.3. Remarks

To date, four *Isospora* spp. are recorded from warblers (Tables 1 and 2). The sizes of the oöcysts of all these species are reasonably compatible with *I. basileuterusi* n. sp.; however, these can be easily distinguished by a few characteristic features: the new species is the only one with ellipsoidal to lemon-shaped sporocysts, small sub-Stieda body and membrane-bound sporocyst residuum. Additionally, *I. basileuterusi* n. sp. does not have the typical characteristics of the other species, such as the absence of polar granules in *Isospora cardellinae* Salgado-Miranda, Medina, Zepeda-Velázquez, García-Conejo, Galindo-Sánchez, Janczur & Soriano-Vargas, 2016 and *Isospora celata* Berto, Medina, Salgado-Miranda, García-Conejo, Janczur, Lopes & Soriano-Vargas, 2014 (see Berto et al., 2014b;

Table 1
Comparative morphological data for oöcysts of *Isospora* spp. recorded from warblers (Parulidae)

Species	Host	Shape	Size (μm)	Shape index	Polar granules	Oöcyst residuum	Wall (μm)	Micropyle	Reference
Isospora basileuterusi Mello & Berto n. sp.	*Basileuterus culicivorus* (Deppe)	Ellipsoidal to ovoidal	22–28 × 17–23 (25.2 × 21.1)	1.1–1.3 (1.20)	Present, 1–3 (usually one)	Absent	1.5–1.9 (1.6)	Absent	Present study
Isospora cardellinae Salgado-Miranda, Medina, Zepeda-Velázquez, García-Conejo, Galindo-Sánchez, Janczur & Soriano-Vargas, 2016	*Cardellina rubra* (Swainson)	Subspherical	23–28 × 23–27 (26.6 × 25.4)	1.0–1.1 (1.1)	Absent	Absent	1.2–1.4 (1.3)	Absent	Salgado-Miranda et al. (2016)
Isospora celata Berto, Medina, Salgado-Miranda, García-Conejo, Janczur, Lopes & Soriano-Vargas, 2014	*Leiothlypis celata* (Say)	Subspherical	27–30 × 25–28 (28 × 26)	1.0–1.1 (1.1)	Absent	Present	1.0–1.3 (1.2)	Absent	Berto et al. (2014)
Isospora orbisreinitas Keeler, Yabsley, Adams & Hernandez, 2014	*Basileuterus rufifrons* (Swainson)	Subspherical to ovoidal	21–28 × 19–25 (24.3 × 22.3)	1.0–1.3 (1.0)	Absent or present, 0–4	Absent	–	Absent	Keeler et al. (2014)
Isospora piacobmi Berto, Flausino, Luz, Ferreira & Lopes, 2010	*Geothlypis aequinoctialis* (Gmelin)	Subspherical to ovoidal	21–26 × 20–24 (23.5 × 21.6)	1.1–1.1 (1.1)	Present, 1	Absent	–	Absent	Berto et al. (2010)

Table 2
Comparative morphological data for sporocysts of *Isospora* spp. recorded from warblers (Parulidae)

Species	Host	Shape	Size (μm)	Shape index	Stieda body (μm)	Sub-Stieda body (μm)	Sporocyst residuum	Reference
Isospora basileuterusi Mello & Berto n. sp.	*Basileuterus culicivorus* (Deppe)	Ellipsoidal to lemon-shaped	14–17 × 8–11 (15.3 × 9.5)	1.4–1.8 (1.61)	Present, knob-like, 0.9–1.1 × 1.7–2.1 (1.0 × 1.8)	Present, trapezoidal, 1.1–1.7 × 2.5–2.9 (1.4 × 2.7)	Granules membrane-bound	Present study
Isospora cardellinae Salgado-Miranda, Medina, Zepeda-Velázquez, García-Conejo, Galindo-Sánchez, Janczur & Soriano-Vargas, 2016	*Cardellina rubra* (Swainson)	Ovoidal	18–20 × 11–13 (19.0 × 12.0)	1.6–1.8 (1.7)	Present, knob-like, (1.1 × 2.4)	Present, trapezoidal to rounded, sometimes with irregular base, (1.8 × 4.5)	Scattered spherules	Salgado-Miranda et al. (2016)
Isospora celata Berto, Medina, Salgado-Miranda, García-Conejo, Janczur, Lopes & Soriano-Vargas, 2014	*Leiothlypis celata* (Say)	Ovoidal	15–20 × 11–14 (18 × 13)	1.4–1.5 (1.4)	Present, knob-like, (1.0 × 2.5)	Present, irregular, barely discernible, (1.5 × 4.0)	Scattered spherules	Berto et al. (2014)
Isospora orbisreinitas Keeler, Yabsley, Adams & Hernandez, 2014	*Basileuterus rufifrons* (Swainson)	Ovoidal	12–19 × 10–14 (16.0 × 11.8)	1.0–1.9 (1.4)	Present, knob-like	Present, prominent, trapezoidal and compartmentalized	Many diffuse granules	Keeler et al. (2014)
Isospora piacobrai Berto, Flausino, Luz, Ferreira & Lopes, 2010	*Geothlypis aequinoctialis* (Gmelin)	Ovoidal	15–17 × 9–12 (15.8 × 10.5)	1.4–1.6 (1.5)	Present, knob-like and prominent, (1.0 × 1.7)	Present, large, trapezoidal and homogeneous, (2.3 × 4.8)	Granules of different sizes	Berto et al. (2010)

Salgado-Miranda et al., 2016), the presence of oöcyst residuum in *I. celata*, the compartmentalized sub-Stieda body in *Isospora orbisreinitas* Keeler, Yabsley, Adams & Hernandez, 2014 and the large and trapezoidal sub-Stieda body in *Isospora piacobrai* Berto, Flausino, Luz, Ferreira & Lopes, 2010 (see Berto et al. (2009); Keeler et al., 2014).

Isospora basileuterusi n. sp. also differs morphologically from the molecularly most closely related *Isospora* spp. (Fig. 3), *Isospora serinuse* Yang, Brice, Elliot & Ryan, 2015 and *Isospora oliveirai* Ortúzar-Ferreira & Berto, 2020. The same characteristics typical of the new species, i.e. ellipsoidal to lemon-shaped sporocysts, small sub-Stieda body and membrane-bound sporocyst residuum, are not observed in *I. serinuse* and *I. oliveirai*.

3.2. Phylogenetic analysis

DNA amplification of the oöcyst of *I. basileuterusi* n. sp. showed a clear band of c.250 bp. Phylogenetic analysis included 18 sequences for avian *Isospora* spp. available on GenBank (Fig. 3). *Toxoplasma gondii* (Nicolle & Manceaux, 1908) was used as the outgroup. *Isospora basileuterusi* n. sp. was recovered in a clade with the highest similarity of 99.5% with *I. serinuse* from island canaries *Serinus canaria* (L.) in Western Australia (Yang et al., 2015). Furthermore, *I. basileuterusi* n. sp. was closely related (95–97%) to *I. oliveirai* from the greenish schiffornis *Schiffornis virescens* (Lafresnaye) in south-eastern Brazil and *Isospora* spp. recovered from thrushes (Turdidae) and tits (Paridae) in Czech Republic (Trefancová & Kvičerová, 2019; Ortúzar-Ferreira et al., 2020).

4. Discussion

Duszynski & Wilber (1997) compiled almost all taxonomic studies of coccidia of passerines and advised that new coccidian identifications should be based on comparative morphology between coccidian species recorded in the same host family. In this sense, the morphotype observed

Fig. 3. Maximum likelihood tree for *Isospora* spp. estimated from the *cox*1 sequences. Numbers at nodes represent bootstrap support (1000 replicates; only values > 50% shown) for Neighbor-Joining and Maximum Likelihood, respectively. The scale-bar represents the number of nucleotide substitutions per site.

from the golden-crowned warblers in this study, *I. basileuterusi* n. sp., was compared with the four recorded coccidian species of Parulidae, as shown in Tables 1 and 2. *Isospora basileuterusi* n. sp. differs in several characteristic features, but can be mainly differentiated from the others by the typical lemon-shape of its sporocysts.

The host of the new coccidian species described here, the golden-crowned warbler *B. culicivorus*, has a wide distribution in the Neotropical region, from Mexico to southern South America (Pacheco et al., 2021). However, according to BirdLife International (2021) this species is the stripe-crowned warbler, which is restricted to Mexico and Central America, not occurring in Brazil. This misinformation is due to species/subspecies status within the genus *Basileuterus* Cabanis. Birdlife have reclassified some subspecies of *B. culicivorus* to the species level, such as *Basileuterus culicivorus auricapilla* (Swainson, 1838) which has been reclassified to the level of species, as *Basileuterus auricapilla* Swainson, 1838. Therefore, this study followed the name listed by the Brazilian Ornithological Records Committee (Pacheco et al., 2021); however, it is noteworthy that the bird specimen in this study is identified as *B. auricapilla* by BirdLife International (2021). Anyway, regardless of the specific identification within *Basileuterus*, due to the wide geographical distributions of warblers (Parulidae) in the Americas, their coccidian species must be equally distributed throughout the Neotropical region.

In the present study, the molecular identification of *I. basileuterusi* n. sp. was performed using the *cox*1 gene, which is considered to be the gene with the highest resolution in detecting recent speciation events (Barta, 2001; Ogedengbe et al., 2011). In fact, the 250 bp *cox*1 gene sequence was not 100% similar to any other deposited on GenBank, contrary to what occurs with ribosomal gene sequences that are more conserved and more suitable for phylogenetic studies of families and orders (Genovez-Oliveira et al., 2020). On the other hand, the region of the *cox*1 sequenced for *I. basileuterusi* n. sp. did not provide conclusive results related to ancestry, as linked to host family, biogeographical region, morphology/biology of the coccidian species, etc. (Fig. 3). This is also observed when *Isospora* spp. amplified and sequenced with the primers (Dolnik et al., 2009) used in the present study are exclusively included in the phylogeny, as in the studies of Yang et al. (2015) and Silva-Carvalho et al. (2018). Perhaps the short sequence of only 250 bp did not allow greater resolution in the phylogenetic study; in this case, sequences with more than 600 bp from other regions of the *cox*1 gene, such as those generated by the JAV primer (Genovez-Oliveira et al., 2020), would show better phylogenetic estimations in the future. Fatally, in the present study these JAV primers were not successful in amplifying the samples; however, it has been shown in any case that mitochondrial genes, such as *cox*1, are better suited to work with individual oöcysts, as the number of copies of mitochondrial DNA is far greater than the number of copies of nuclear DNA, thus favoring the amplification of mitochondrial genes (Dolnik et al., 2009).

5. Conclusion

The comparison of *I. basileuterusi* n. sp. with *Isospora* spp. described from Neotropical warblers clearly supports the designation as a unique species. Therefore, *I. basileuterusi* is considered as new to science, which is the fifth species described in a host of the family Parulidae and the first molecularly characterized *via* sequencing the *cox*1 gene.

Funding

This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro. MSO has a post-doctoral scholarship from FAPERJ (Grant/Award Number: E–26/204.228/2021). LASA has a scholarship from CAPES (Grant/Award Number: 001). BPB has fellowships from CNPq (Grant/Award Number: 303899/2019-0) and from FAPERJ (Grant/Award Number: E–26/202.797/2019).

Ethical approval

Field-collecting permits were issued by SISBIO/ICMBio (licenses 45,200; 49,605; 54,951; 70,132), CEUA/UFRRJ (protocols IV-036/2014; ICBS-008/2015; IV-6606250616) and CEUA/UNIGRANRIO (protocol 021/2019). All applicable institutional, national and international guidelines for the care and use of animals were followed.

CRediT author statement

The study was designed by SVC, VML and BPB. Field work was performed by MSO, LASA and BPB. Laboratory procedures for maintenance, recovery, measurements, photomicrographs and isolation of oöcysts were performed by MSO and LASA. DNA extraction, amplification and sequencing were performed by ERM, AAO and VML. BPB analyzed the data and drew the coccidian oöcyst. The manuscript was written by ERM and BPB and subsequently revised by all other authors. All authors read and approved the final manuscript.

Data availability

Photosyntypes, line drawing, and oöcysts in 70% ethanol are deposited and available (http://r1.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under the repository number P-124/2021, along with the photographs of the type-host specimen (symbiotype). The generated sequence for *I. basileuterusi* n. sp. is deposited in the GenBank database under the accession number OM025014.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are thankful to staff at the Parque Nacional do Itatiaia, mainly to the research coordinator Dr Léo Nascimento, that allowed us to access and use some facilities during the expeditions.

References

Barta, J.R., 2001. Molecular approaches for inferring evolutionary relationships among protistan parasites. Vet. Parasitol. 101, 175–186.

Berto, B.P., Luz, H.R., Flausino, W., Ferreira, I., Lopes, C.W.G., 2010. *Isospora piacobrai* n. sp. (Apicomplexa: Eimeriidae) from the masked yellowthroat *Geothlypis aequinoctialis* (Gmelin) (Passeriformes: Parulidae) in South America. Syst. Parasitol. 75, 225–230.

Berto, B.P., McIntosh, D., Lopes, C.W.G., 2014a. Studies on coccidian oocysts (Apicomplexa: Eucoccidiorida). Rev. Bras. Parasitol. Vet. 23, 1–15.

Berto, B.P., Medina, J.P., Salgado-Miranda, C., García-Conejo, M., Janczur, M.K., Lopes, C.W.G., Soriano-Vargas, E., 2014b. *Isospora celata* n. sp. (Apicomplexa: Eimeriidae) from the orange-crowned warbler *Oreothlypis celata* (Say) (Passeriformes: Parulidae) in Mexico. Syst. Parasitol. 89, 253–257.

Berto, B.P., Lopes, C.W.G., 2020. Coccidia of wild birds as ecological biomarkers: Some approaches on parasite-host-environment interaction. J. Parasitol. 106, 707–713.

BirdLife International, 2021. IUCN Red List for birds. Available from: http://www.birdlife.org. (Accessed 22 December 2021).

Dias, B.F.S., 1992. Alternativas de Desenvolvimento dos Cerrados: Manejo e Conservação dos Recursos Naturais Renováveis. Fundação Pró-Natureza, Brasília.

Dolnik, O.V., Palinauskas, V., Bensch, S., 2009. Individual oocysts of *Isospora* (Apicomplexa: Coccidia) parasites from avian feces: from photo to sequence. J. Parasitol. 95, 169–174.

Duszynski, D., Wilber, P., 1997. A guideline for the preparation of species descriptions in the Eimeriidae. J. Parasitol. 83, 333–336.

Genovez-Oliveira, J.L., Oliveira, M.S., Thode-Filho, S., Cardozo, S.V., Oliveira, A.A., Lima, V.M., et al., 2020. Morphological and molecular identification of *Isospora massardi* Lopes, Berto, Luz, Galvão, Ferreira & Lopes, 2014 (Chromista: Miozoa: Eimeriidae) from thrushes *Turdus* spp. (Passeriformes: Turdidae) in South America. Parasitol. Int. 75, 102040.

ICMBIO, 2021. Parque Nacional do Itatiaia. Available from: http://www.icmbio.gov.br/parnaitatiaia. (Accessed 22 December 2021).

ICZN, 2012. International Commission on Zoological Nomenclature: Amendment of Articles 8, 9, 10, 21 and 78 of the International Code of Zoological Nomenclature to expand and refine methods of publication. Bull. Zool. Nomencl. 69, 161–169.
Keeler, S.P., Yabsley, M.J., Adams, H.C., Hernandez, S.M., 2014. A novel *Isospora* species (Apicomplexa: Eimeriidae) from warblers (Passeriformes: Parulidae) of Costa Rica. J. Parasitol. 100, 302–304.
Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549.
Lima, A.L.D.C., Manhães, M.A., 2009. Hábitos alimentares de *Basileuterus culicivorus* (Aves: Parulidae) em uma área de Mata Atlântica secundária, sudeste do Brasil. Biota Neotropica 9, 137–143.
Marini, M.A., Cavalcanti, R.B., 1993. Habitat and foraging substrate use of three *Basileuterus* warblers from central Brazil. Ornitol. Neotrop. 4, 69–76.
Ogedengbe, J.D., Hanner, R.H., Barta, J.R., 2011. DNA barcoding identifies *Eimeria* species and contributes to the phylogenetics of coccidian parasites (Eimeriorina, Apicomplexa, Alveolata). Int. J. Parasitol. 41, 843–850.
Ortúzar-Ferreira, C.N., Genovez-Oliveira, J.I., Oliveira, M.S., Mello, E.R., Thode-Filho, S., Oliveira, Á.A., et al., 2020. *Isospora oliveirai* n. sp. (Chromista: Miozoa: Eimeriidae) from the greenish schiffornis *Schiffornis virescens* (Lafresnaye, 1838) (Passeriformes: Tyranni: Tityridae) in South America. Acta Parasitol. 65, 843–851.
Pacheco, J.F., Silveira, L.F., Aleixo, A., Agne, C.E., Bencke, G.A., Bravo, G.A., et al., 2021. Annotated checklist of the birds of Brazil by the Brazilian Ornithological Records Committee - second edition. Ornithol. Res. 29, 94–105.
Salgado-Miranda, C., Medina, J.P., Zepeda-Velázquez, A.P., García-Conejo, M., Galindo-Sánchez, K.P., Janczur, M.K., Soriano-Vargas, E., 2016. *Isospora cardellinae* n. sp. (Apicomplexa: Eimeriidae) from the red warbler *Cardellina rubra* (Swainson) (Passeriformes: Parulidae) in Mexico. Syst. Parasitol. 93, 825–830.
Sick, H., 1997. Ornitologia Brasileira. Nova Fronteira, Rio de Janeiro.
Silva-Carvalho, L.M., Pastura, D.G.N., Gomes, J.V., Siqueira, P.B., Rodrigues, M.B., Lima, V.M., Berto, B.P., 2018. *Isospora lopesi* n. sp. (Apicomplexa: Eimeriidae) from the eastern white-throated spadebill *Platyrinchus mystaceus* Vieillot (Passeriformes: Tyranni: Tyrannidae) in South America. Syst. Parasitol. 95, 455–463.
Trefancová, A., Kvičerová, J., 2019. *Isospora svecica* sp. n. (Apicomplexa: Eimeriidae), a new species of coccidium from the white-spotted bluethroat *Luscinia svecica cyanecula* (Aves: Passeriformes: Muscicapidae). Parasitol. Res. 118, 3043–3051.
Williams, R.B., Thebo, P., Marshall, R.N., Marshall, J.A., 2010. Coccidian oöcysts as type-specimens: long-term storage in aqueous potassium dichromate solution preserves DNA. Syst. Parasitol. 76, 69–76.
Yang, R., Brice, B., Elliot, A., Ryan, U., 2015. *Isospora serinuse* n. sp. (Apicomplexa: Eimeriidae) from a domestic canary (*Serinus canaria* forma domestica) (Passeriformes: Fringillidae) in Western Australia. Exp. Parasitol. 159, 59–66.

Parasitology Research (2024) 123:42
https://doi.org/10.1007/s00436-023-08045-5

RESEARCH

Molecular and statistical approaches to the delimitation of Eimeriidae species: a case of extreme polymorphism in eimerian oocysts from the plumbeous pigeon *Patagioenas plumbea* (Vieillot, 1818) (Columbiformes) in South America

Carlos Nei Ortúzar-Ferreira[1] · Mariana S. Oliveira[1] · Lucas de Assis S. Andrade[1] · Ericson R. de Mello[2] · Viviane M. Lima[3] · Bruno P. Berto[3]

Received: 10 May 2023 / Accepted: 3 November 2023 / Published online: 14 December 2023

Abstract

The current work aimed to analyze, morphologically, statistically, and molecularly, oocysts shed from plumbeous pigeons, *Patagioenas plumbea* (Vieillot, 1818), from a locality at 2197 m of altitude near the Agulhas Negras peak, the highest point of the State of Rio de Janeiro, southeastern Brazil. The oocysts were extremely polymorphic, being subspheroidal, ovoidal, or ellipsoidal, in addition to having the random presence/absence of characteristic features associated with the oocyst wall, such as micropyle, micropyle cap, lateral micropyle, and outer veil/rough wall. Linear regression confirmed the extreme polymorphism of oocysts, showing that if all combinations of taxonomic characters in oocysts (morphotypes) were overestimated, 19 different species could be identified/described. In contrast, the means comparison analysis between oocysts with the presence/absence of characteristic features and the histograms showed equivalences and regularity in the distribution in the classes of measures, which indicate the presence of a single species in the measured oocysts. Molecular analyses were performed from the isolation of individual oocysts of different morphotypes, which had their genetic material extracted, amplified, and sequenced in 4 non-overlapping *loci* in the *cox1* and *cox3* genes and fragments of the small and large subunit rDNA of mitochondrial DNA. The sequences were 100% identical between the morphotypes, with the exception of a very small divergence observed at the *locus* that partially covers the *cox3* gene. The phylogenetic analysis was inconclusive for the *locus* within the *cox1* gene traditionally used for eimeriid coccidians; however, the other *loci* should have a promising future for phylogenetic studies when more sequences for the same genic regions are deposited in GenBank. Finally, the multifactorial analysis of the current work supported that the polymorphic oocysts shed from *P. plumbea* are a single species, which was named *Eimeria patagioenasae*, making this the twenty-second eimerian description from Columbiformes.

Keywords *Eimeria patagioenasae* · Coccidia · Oocysts · Phylogeny · Neotropical birds · Parque Nacional de Itatiaia

Section Editor: Berit Bangoura

✉ Mariana S. Oliveira
mariana.s.o@hotmail.com

✉ Bruno P. Berto
bertobp@ufrrj.br

[1] Programa de Pós-Graduação em Biologia Animal, Instituto de Ciências Biológicas e da Saúde (ICBS), Universidade Federal Rural do Rio de Janeiro (UFRRJ), BR-465 Km 7, Seropédica, RJ 23897-000, Brazil

[2] Programa de Pós-Graduação em Ciências Veterinárias, Instituto de Veterinária, UFRRJ, BR-465 Km 7, Seropédica, RJ 23897-000, Brazil

[3] Departamento de Biologia Animal, ICBS, UFRRJ, BR-465 Km 7, Seropédica, RJ 23897-000, Brazil

Introduction

Knowledge of the parasite diversity of wild animals is quite scarce when compared to what is known about the diversity of parasites in domestic animals and humans. In a recent study, Duszynski (2021) points out that of the current diversity of vertebrates, only a very small portion was examined for coccidians. Also, Ortúzar-Ferreira et al. (2020) showed at the time that of the 367 species of columbiform birds in the world, only 15 were reported as coccidian hosts.

Pigeons and doves (Columbiformes) are hosts for a wide range of parasites, from ectoparasites such as hippoboscids, mallophages, lice, and mites to endoparasites such as

helminths, hemoprotozoans, and coccidians (Atkinson et al. 2008; Taylor et al. 2017). In this sense, Ortúzar-Ferreira et al. (2020) provided a taxonomic review of eimeriid coccidians from Columbiformes, where 19 *Eimeria* spp. and 2 *Isospora* spp. are recorded. Since then, two new descriptions of *Eimeria* spp. have been added: *Eimeria chalcoptereae* Yang et al. 2020 and *Eimeria ferreirai* (Oliveira and Berto 2020; Oliveira et al. 2020; Yang et al. 2020). Therefore, currently, a total of 21 *Eimeria* spp. are recorded from columbiform birds.

Based on this, the current work examined fecal samples of wild birds in a locality at 2197 m of altitude near the Agulhas Negras peak, the highest point of the State of Rio de Janeiro. Oocysts with different shapes and irregular characteristic features were observed from plumbeous pigeons, *Patagioenas plumbea* (Vieillot, 1818), which is a columbiform species with medium forest dependency and related to well-preserved environments in South America, and in southeastern Brazil, mainly observed on the slopes of mountainous regions (Sick 1997; Mello et al. 2020; BirdLife International 2023). A detailed morphological study of these polymorphic oocysts is provided here, associated with statistical and molecular studies, aiming at the specific identification of these oocysts.

Materials and methods

Sample collection

A single expedition was conducted in the high-altitude plateau of the Itatiaia National Park, a protected area with a high degree of vulnerability located in the Serra da Mantiqueira on the border of the States of Rio de Janeiro, Minas Gerais, and São Paulo (ICMBIO 2023). This expedition took place in November 2021, specifically at km 13 of the "Travessia Ruy Braga" (Ruy Braga Crossing) (22°24′29.97″S; 44°39′04.07″W), which is the trail that leads to the high altitude plateau where the Agulhas Negras peak is located. Mist nets were used to capture the birds. Three plumbeous pigeons, *P. plumbea*, were captured. The captured birds were specifically identified (Ridgely et al. 2015; Mello et al. 2020), photographed, and banded with numbered metal rings provided by the Brazilian bird-ringing agency (Centro Nacional de Pesquisa e Conservação de Aves Silvestres (CEMAVE). Subsequently, the birds were kept in individual boxes lined with clean paper until defecation, when they were released at the same place of capture. Each fresh droplet of feces from each individual bird was placed individually in a centrifuge tube with a potassium dichromate 2.5% ($K_2Cr_2O_7$) solution (Dolnik 2006).

Morphological analyses

Samples were examined at the Laboratório de Biologia de Coccídios, Universidade Federal Rural do Rio de Janeiro (UFRRJ). All samples were incubated at room temperature (25 °C) for 7 days. Forty oocysts were isolated by flotation in Sheather's sugar-saturated solution (specific gravity: 1.20) and examined microscopically using the technique described by Duszynski and Wilber (1997) and Berto et al. (2014). Morphological observations, line drawings, photomicrographs, and measurements were made using an Olympus BX binocular microscope (Olympus Optical, Tokyo, Japan) equipped with a digital camera Eurekam 5.0 (BEL Photonics, Monza, Italy). Line drawings, photomicrographs, and other figures were edited using two software applications (Corel DRAW and Corel PHOTO-PAINT) from CorelDRAW® (Corel Draw Graphics Suite, Version 2020, Corel Corporation, Canada). All measurements were made in micrometers and are presented as the range followed by the mean in parentheses.

Statistical analyses

Three statistical methods were employed after the previous evaluation of the data by D'Agostino's test of normality: (1) Analysis of variance (ANOVA) was used to compare measurements of the length, width, and length/width (L/W) ratio of the oocysts and sporocysts with different characteristic features, using the statistical package Biostat 5.0 (Ayres et al. 2007) to calculate the mean, variance, degree of freedom, and p value (Sampaio 2002; Berto et al. 2014); (2) histograms were prepared to plot the values of length, width and the L/W ratio of the oocysts, as well as their relative frequencies, according to the methods of Sampaio (2002) and Berto et al. (2014); and (3) linear regression to determine the distribution of oocysts using methods proposed by Norton and Joyner (1981) and subsequently modified by Berto et al. (2014). The graphs, line regression, data points, the R^2 (coefficient of determination) value, and the coefficient of the regression line were obtained using the software Microsoft Excel 2007® (Microsoft, Redmond, Washington).

Molecular analyses

Individual oocysts, morphologically analyzed in detail and photomicrographed under light microscopy, were isolated and resuspended in 90 µl of PBS (i.e., every single oocyst with analyzed morphology was contained in each PBS suspension), according to the guidelines of Dolnik et al. (2009). Four freeze–thaw cycles (−4 °C and 96 °C, respectively) of 5 min each were applied to break the

oocyst wall before the DNA extraction to achieve complete oocyst lysis. After this step, 10 µl of proteinase K was added, leaving it to incubate in a water bath at 56 °C for approximately 2 h. Complete DNA extraction was performed using the Qiagen DNeasy™ Blood and Tissue Kit (Qiagen, São Paulo, Brazil) according to the manufacturer's instructions. PCR amplification of four different (non-overlapping) *loci* of the mitochondrial genome was targeted (Fig. 1). Three of these *loci* were designed using Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/), and the fourth was the COIBF1 *locus*, primarily developed by Dolnik et al. (2009) and extensively applied to eimeriid coccidians since then (Table 1). For amplification, a 25-µl PCR reaction was prepared using 3 µl of genomic DNA (< 1 µg), 12.5 µl of GoTaq® G2 Hot Start Colorless Master Mix (Promega Labs) (1X), 0.25 µl of each Primer (0.2 µM) and 9 µl of nuclease-free water. PCR amplifications were conducted using the cycling conditions shown in Table 1. Amplicons from the nested PCRs were purified using the Qiagen MinElute™ PCR Purification (Qiagen, São Paulo, Brazil).

DNA sequence analyses

All PCR products were sequenced using the PCR forward and reverse primers by Ludwig Biotechnology, where an ABI-Prism 3500 Genetic Analyzer (Applied Biosystems, Foster City, California) was used for Sanger sequencing. The results of the sequencing reactions were analyzed and edited in the Chromas 2.6 program. Sequences were compared with other coccidian parasites available on the GenBank database using the Basic Local Alignment Search Tool (BLAST). Alignments were created in MEGA v10.2.6 using Clustal W (http://www.clustalw.genome.jp). Phylogenetic relationships were reconstructed using Bayesian Inference in the MrBayes v3.2.7 (Ronquist et al. 2012) and the maximum likelihood method in MEGA (Kumar et al. 2018). The best-fitting evolutionary models for all phylogenetic analyses were selected by the Model Selection in MEGA. Bayesian Inference analysis was conducted under the GTR + G evolutionary model for 1,000,000 generations, and the trees were summarized after removing 25% of burn-in. Maximum likelihood analysis was conducted under the TN93 + G evolutionary model, and the bootstrap values were calculated by 1,000

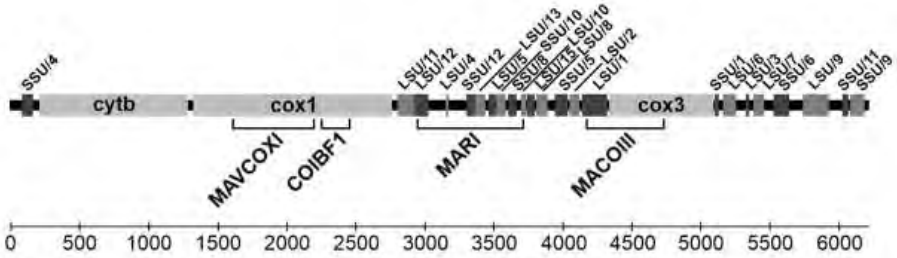

Fig. 1 Mitochondrial genome organization of *Eimeria* spp. from birds (based on the complete mitochondrial genome of *Eimeria dispersa* available on GenBank/BLAST under accession number KJ608416): the *loci* MAVCOXI (positioned between ~ 1600 and 2200 nt) and COIBF1 (positioned between ~ 2250 and 2500 nt) occupy the gene *cox1*; the MARI *locus* (positioned between ~ 2950 and 3750 nt) occupies fragments of small subunit (SSU) and large subunit (LSU) rDNA, and the MACOIII *locus* (positioned between ~ 4200 and 4750 nt) occupies the LSU/1 rDNA fragment and the *cox3* gene

Table 1 Primers and cycling conditions for PCR amplification of four *loci* of the mitochondrial genome

Locus	Primer	Primer sequence	Cycling conditions
COIBF1	COIBf1 (external; forward)	5′-GWTCATTAGTATGGGCACATCA-3′	35 cycles of 94 °C for 30 s, 45 °C for 45 s, 72 °C for 30 s, and a final extension of 72 °C for 5 min
	COIBr1 (external; reverse)	5′-CCAAGAGATAATACRAARTGGAA-3′	
	COIBf2 (internal; forward)	5′-GGGCACATCATATGATGAC-3′	
	COIBr2 (internal; reverse)	5′-ATAGTATGTATCATGTARWGCAA-3′	
MACOIII	MACOIIIf (forward)	5′-CTGTAGAGTCGAGATGGAAACAA-3′	35 cycles of 94 °C for 30 s, 50 °C for 45 s, 72 °C for 42 s, and a final extension of 72 °C for 5 min
	MACOIIIr (reverse)	5′-CCTAGAATAACACTGGCTGTAGATAG-3′	
MAVCOXI	MAVCOXIf (forward)	5′-GCCAGGTCTATATGGTGGATATG-3′	35 cycles of 94 °C for 30 s, 51 °C for 45 s, 72 °C for 54 s, and a final extension of 72 °C for 5 min
	MAVCOXIr (reverse)	5′-TGCCCAGACTAATGAACCTAAC-3′	
MARI	MARIf (forward)	5′-GCTCATCACACCCTTGTACTT-3′	35 cycles of 94 °C for 30 s, 51 °C for 45 s, 72 °C for 54 s, and a final extension of 72 °C for 5 min
	MARIr (reverse)	5′-CCCGGCTAAACTTCCCTTATT-3′	

replicates. The resultant phylogenetic trees were visualized in MrBayes and MEGA and exported in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/).

Results

Prevalence and description

Three plumbeous pigeons (*P. plumbea*) were examined, and two were positive for coccidian oocysts. These oocysts were polymorphic in size, shape, and the presence/absence of certain characteristic features; however, in any case, all morphotypes were distinct from those coccidial species recorded as parasites of Columbiformes (Table 2). This material is described below.

Eimeria patagioenasae Ortúzar-Ferreira & Berto n. sp. (Figs. 2 and 3)
Kingdom: Chromista Cavalier-Smith, 1981
Phylum: Miozoa Cavalier-Smith, 1987
Infraphylum: Apicomplexa Levine, 1970
Class: Coccidiomorphea Doflein, 1901
Subclass: Coccidia Leuckart, 1879
Family: Eimeriidae Minchin, 1903
Genus: *Eimeria* Schneider, 1875

Oocyst ($n=40$) subspheroidal (18%), ovoidal (45%), or ellipsoidal (37%): 21–31 × 16–23 (26.1 × 20.1); L/W ratio: 1.0–1.6 (1.31). Wall bi-layered, 1.5–2.0 (1.8 µm) thick, outer layer smooth, *c.*2/3 of total thickness. Some oocysts (38%) had a wrinkled membrane (probably the outer veil) adhered to the outer layer of the oocyst wall, leading to a rough appearance of the oocyst wall. Micropyle present at the longitudinal end of most oocysts (78%), 2.0–9.2 (4.7) wide; randomly associated with a micropyle cap (48%), 0.8–2.7 high × 4.0–10.5 wide (1.6 × 7.1). A lateral micropyle was observed in some oocysts (35%), 3.9–8.9 (6.6) wide. Oocyst residuum is absent, but 1–2 polar granules (usually 1) are present: 2.3–3.1 × 1.3–2.4 (2.7 × 1.8). Sporocyst ($n=27$) elongate ellipsoidal to fusiform: 13–18 × 6–8 (15.0 × 7.4); L/W ratio: 1.8–2.3 (2.04). Stieda body present, flattened to knob-like, barely discernible from the sub-Stieda body in some sporocysts, 0.4–0.9 high × 0.9–1.5 wide (0.6 × 1.2). Sub-Stieda body present, rounded to trapezoidal, 0.8–1.4 high × 1.3–2.2 wide (1.0 × 1.8). Para-Stieda body absent. Sporocyst residuum is present, consisting of numerous granules scattered among the sporozoites. Sporozoites vermiform, with posterior and anterior refractile bodies, 3.4–5.9 × 2.4–3.3 (4.4 × 2.8), and a central nucleus.

Diagnosis

Eimeria patagioenasae is the only coccidian species recorded from the host species *P. plumbea*. The oocysts of *E. patagioenasae* are primarily identifiable by the random presence of micropyle, micropyle cap, lateral micropyle, and outer veil/rough wall, which may be subspheroidal, ovoid, or ellipsoidal, in addition to the characteristic features typical of the fusiform sporocysts with Stieda and sub-Stieda bodies (Figs. 2 and 3). Based on these and other taxonomic characters, differences between *E. patagioenasae* and other *Eimeria* spp. recorded from Columbiformes are summarized in Table 2. Of the 21 coccidian species recorded from Columbiformes to date, only *Eimeria turturi* Golemansky 1976, has a shape (L/W ratio mean of 1.3) and size compatible with *E. patagioenasae*; however, this species is easily differentiated by the absence of micropyle and polar granule, in addition to the shape, size, and other characteristic features of the sporocysts. *Eimeria kapotei* Chatterjee and Ray 1969; *Eimeria waiganiensis* Varghese 1978; *Eimeria duculai* Varghese 1980; *Eimeria palumbi* McQuistion 1991; *Eimeria zenaidae* Adriano et al. 2003; *Eimeria janovyi* Bandyopadhyay et al. 2006; *Eimeria columbapalumbi* Jamriška and Modrý, 2012; *Eimeria lyoni* Yabsley et al. 2015; and *E. chalcoptereae* are slightly similar in size (but not shape) to *E. patagioenasae*, in addition to being easily differentiated by the absence of micropyle and/or other characteristic features of sporocysts. *Eimeria ferreirai* is slightly similar to *E. patagioenasae*, which are sympatric (hosts and coccidians) in Itatiaia National Park; however, *E. patagioenasae* can be differentiated, in addition to size and shape, by micropyles randomly present, sporocyst shape, and Stieda and sub-Stieda bodies slightly distinct, while *E. ferreirai* has only a well-defined Stieda body as an excystment structure.

Taxonomic summary

Type host: *Patagioenas plumbea* (Vieillot, 1818) (Aves: Columbiformes: Columbidae), plumbeous pigeon.

Type locality: high altitude plateau of the Itatiaia National Park (22°24′29.97″S; 44°39′04.07″W), Southeastern Brazil.

Type-material: photosyntypes, line drawing and oocysts in 2.5% $K_2Cr_2O_7$ solution (Williams et al. 2010) are deposited and available (http://r1.ufrrj.br/labicoc/colecao.html) in the Parasitology Collection of the Laboratório de Biologia de Coccídios, at UFRRJ, under the repository number P-132/2023. Photographs of the type-host specimen (symbiotype) are deposited in the same collection.

Representative DNA sequence: DNA amplification of the COIBF1, MACOIII, MAVCOXI and MARI *loci* showed clear bands around ~ 250 bp, ~ 632 bp, ~ 653 pb, and ~ 824 bp, respectively (Fig. 4). Representative sequences were deposited in the GenBank database under the accession numbers:

Table 2 Comparative morphology of *Eimeria* spp. recorded from Columbiformes of the World

Coccidia	Hosts	Locality	References	Oocysts								Sporocysts					
				Shape	Length (μm)	Width (μm)	L/W ratio	Wall	Residuum	Micropyle	Polar granule	Shape	Length (μm)	Width (μm)	L/W ratio	Stieda body	Substieda body	Residuum
Eimeria labbeana (Labbe, 1896) Pinto 1928	*Columba livia* Gmelin, 1789	Asia, India	Pinto (1928)	Subspheroidal to ovoidal	17–21	16–18	1.0–1.1	Smooth	Absent	Present	Present	Ovoidal	11–14 (12.4)	5–7 (6.4)	(1.4)	–	–	Present
			Nieschulz (1935)	Subspheroidal to ellipsoidal	15–18 (16.7)	14–16 (15.3)	1.0–1.1 (1.09)	Smooth	Absent	Absent	Present	Ellipsoidal	–	–	–	Present	Absent	Present
	Streptopelia decaocto Frivaldszky, 1838	Europe, Portugal	Oliveira et al. (2021)	Subspheroidal to ellipsoidal	16–21 (18.5)	14–17 (15.5)	1.0–1.4 (1.19)	Smooth	Absent	Present, 1–3	Present, 1–3	Ovoidal or slightly reniform	11–14 (12.2)	5–7 (5.9)	1.9–2.3 (2.08)	Present, prominent triangular	Absent	Present
	Columba palumbus Linnaeus, 1758																	
Eimeria columbarum Nieschulz 1935	*C. livia*	Asia, India	Nieschulz (1935)	Subspherical to ovoidal	19–21 (20)	17–20 (18.7)	1.0–1.1 (1.07)	Smooth	Absent	Absent	Present	Ellipsoidal	–	–	–	Present	Absent	Present
Eimeria columbae Mitra and Das-Gupta 1937	*C. livia*	Asia, India	Mitra and Das Gupta (1937)	Subspherical to ovoidal	(16.4)	(14.4)	–	–	Present	Absent	–	Ellipsoidal	(7.2)	(4.8)	–	–	–	Present
Eimeria spheruceruae Ray 1952	*Treron sphenurus* (Vigors, 1832)	Asia, India	Ray (1952)	Rhiniform to ellipsoidal	17–25 (19.2)	12–15 (12.6)	–	Smooth with a lateral dent	Absent	Present, asymmetrical, with a micropyle cap	–	Broadly ovoidal	17–19 (17.5)	12–14 (12.5)	–	–	–	Present
Eimeria kapotei Chatterjee and Ray 1969	*C. livia*	Asia, India	Chatterjee and Ray (1969)	Subspherical	24 – 30 (26.1)	22 – 26 (23.5)	–	–	–	Present, anterior	Present, 1–2	Ovoidal	8 – 10	–	–	Present	–	Present, scattered
Eimeria turturi Golemansky 1976	*Streptopelia turtur* (Linnaeus, 1758)	Europe, Bulgaria	Golemansky (1976)	Ellipsoidal or broadly ovoidal	23–29 (26)	18–25 (21.6)	–	Smooth, ~ 1.5	Absent	Absent	Absent	Elongate ellipsoidal	11–13	6–8	–	Absent	–	Present, scattered
Eimeria waigamiensis Varghese 1978	*Chalcophaps indica* (Linnaeus, 1758); *Ducula spilorrhoa* (Gray, 1858)	Oceania, Papua New Guinea	Varghese (1978)	Broadly ovoidal	22–25 (24)	19–23 (22)	1.1–1.2 (1.1)	Smooth, ~ 1.5	Absent	Present, 4–6 (5)	Present, 2–4	Ovoidal	9–11 (10.0)	6–8 (7.0)	–	Present, prominent	Present, small	Present, scattered
Eimeria duculai Varghese 1980	*Ducula spilorrhoa* (Gray, 1858)	Oceania, Papua New Guinea	Varghese (1980)	Broadly ovoidal	26–31 (28)	23–27 (25)	(1.1)	Smooth, 1.5–2.5 (2.0)	Absent	Inconspicuous	Present, 1, ~ 2.0	Elongate	14–16 (13.5)	6–8 (7.2)	–	Present, prominent, conical	Absent	Present, compacted, membrane-bounded
Eimeria gourai Varghese 1980	*Goura victoria* (Fraser, 1844)	Oceania, Papua New Guinea	Varghese (1980)	Subspherical	19–22 (20)	18–21 (20)	(1.0)	Smooth, ~ 1.0	Absent	Absent	Present, 1, ~ 2.0	Elongate	10–13 (12.0)	4–6 (5.5)	–	Present	Absent	Present, compacted
Eimeria palumbi McQuistion 1991	*Zenaida galapagoensis* Gould, 1841	South America Ecuador, Galápagos Islands	McQuistion (1991)	Ovoidal to ellipsoidal	22–27 (24.2)	19–24 (21.7)	1.0–1.2 (1.16)	Smooth, ~ 2.0	Present, round to splinter-like granules	Absent	Absent	Ellipsoidal	15–17 (15.3)	8–9 (8.1)	1.8–2.1 (1.9)	Present, nipple-like	Absent	Present, scattered
Eimeria curvata Adriano et al. 2000	*Columbina talpacoti* (Temminck, 1809); *Columbina squammata* (Lesson, 1831)	South America, Brazil	Adriano et al. (2000)	Ovoidal to ellipsoidal	17–19 (18.3)	15–17 (15.5)	1.1–1.3 (1.2)	Smooth, ~ 1.3	Absent	Absent	Present	Elongate	11–13 (12.3)	5–6 (5.8)	2.0–2.2 (2.1)	Present, protuberant, nipple-like	Absent	Present, compacted
Eimeria zenaidae Adriano et al. 2003	*Zenaida auriculata* (Des Murs, 1847)	South America, Brazil	Adriano et al. (2003)	Subspherical	22–26 (23.8)	19–22 (20.3)	(1.2)	Rough, ~ 1.7	Absent	Absent	Present, 1	Elongate	12–14 (13.1)	7–8 (7.4)	1.7–1.9 (1.8)	Present, large	Absent	Present, scattered

Table 2 (continued)

Coccidia	Hosts	Locality	References	Oocysts								Sporocysts						
				Shape	Length (µm)	Width (µm)	L/W ratio	Wall	Residuum	Micropyle	Polar granule	Shape	Length (µm)	Width (µm)	L/W ratio	Stieda body	Substieda body	Residuum
Eimeria jasoriyi Bandyopadhyay et al. 2006	*C. livia*	Asia, India	Bandyopadhyay et al. (2006)	Ellipsoidal	(24.3)	(19.8)	(1.2)	Smooth, ~1.1	Absent	Absent	Present, 1, subspherical	Pyriform	(12.1)	(10.1)	(1.2)	Present, large, prominent	Absent	Present, scattered
Eimeria livialis Alyousif et al. 2009	*C. livia*	Asia, Saudi Arabia	Alyousif et al. (2009)	Elongate ellipsoidal	19–23 (21)	14–17 (15)	(1.2)	Smooth,1–1.3 (1.2)	Present, irregular globules	Absent	Absent	Ellipsoidal	9–12 (10.6)	6–8 (6.7)	(1.6)	Present, small, nipple-like	Absent	Present, scattered
Eimeria columbapalumbi Jamriška and Modrý 2012	*Columba palumbus* (Linnaeus, 1758)	Europe, Czech and Slovak Republics	Jamriška and Modrý (2012)	Ellipsoidal	17–24 (21.3)	15–18 (16.9)	1.0–1.4 (1.26)	Smooth, 0.6–1.5 (0.9)	Absent	Absent	Present, 2 irregular, ~2.0	Elongate ovoidal, slightly asymmetrical	11–16 (13.5)	6–7 (6.5)	1.7–2.2 (1.9)	Present	Absent	Present, scattered
Eimeria mauritiensis Ball et al. 2012	*Nesoenas mayeri* (Prévost, 1843)	Africa, Madagascar	Ball et al. (2012)	Subspherical	18–22 (19.7)	16–19 (17.8)	1.0–1.2 (1.1)	Smooth, ~0.8	Absent	Absent	Absent	–	8–14 (12.0)	6–7 (6.6)	–	Present	Present	Present
Eimeria lysoni Yabsley et al. 2015	*Zenaida macroura* (Linnaeus,1758)	North America, USA	Yabsley et al. (2015)	Subspherical to ovoidal	23–26 (24.2)	20–22 (20.7)	1.1–1.3 (1.2)	Smooth, ~1.0	Absent	Absent	Present, 1–2	Ovoidal	12–14 (12.4)	7–8 (7.3)	1.5–1.9 (1.7)	Present, knob-like	Present, rounded	Present, scattered, granules of ~1.0
Eimeria sp. of Yang et al. (2016)	*C. livia*	Oceania, Australia	Yang et al. (2016)	Subspherical	19–22 (20.2)	16–19 (16.1)	(1.38)	Smooth, ~1.0	Present	Absent	Present	Elongate ovoidal	12–15 (13.0)	5–7 (6.1)	2.0–2.2 (2.1)	Present	Absent	Present, compacted
Eimeria columbinae Ortúzar-Ferreira & Berto, 2019	*C. talpacoti*	South America, Brazil	Ortúzar-Ferreira et al. (2020)	Subspherical to ellipsoidal	13–16 (14.7)	12–14 (13.2)	1.0–1.2 (1.11)	Smooth, 1.0–1.2 (1.1)	Present, granules bonded and/or diffused	Inconspicuous	Absent	Ellipsoidal to slightly asymmetrical	8–10 (9.0)	5–6 (5.1)	1.6–2.0 (1.77)	Present, flattened to half-moon-shaped	Present, rounded	Present, scattered
Eimeria chalcopterosae Yang et al. 2020	*Phaps chalcoptera* (Latham 1790)	Oceania, Australia	Yang et al. (2020)	Subsphenoidal	22–25 (23.5)	21–24 (22.6)	1.0–1.1 (1.04)	Smooth, 1.0–1.4 (1.2)	Absent	Barely discernible	Present, 2–3	Ellipsoidal	13–14 (13.5)	7–8 (7.2)	1.8–2.0 (1.88)	Present, flattened to half-moon-shaped	Present, rounded to trapezoidal	Present, membrane-bounded granules
Eimeria ferreirai Oliveira & Berto, 2020	*Leptotila verreauxi* Bonaparte, 1855; *Leptotila rufaxilla* (Richard & Bernard, 1792)	South America, Brazil	Oliveira et al. (2020)	Subspherical to ellipsoidal	19–25 (21.4)	16–21 (18.8)	1.0–1.4 (1.15)	Smooth, 1.3–1.9 (1.6)	Absent	Present, with a micropyle cap barely discernible	Present, 1–2	Elongate ovoidal to boomerang-shaped	12–15 (13.4)	6–8 (6.9)	1.8–2.2 (1.95)	Present, triangular to lozengal	Absent	Present, scattered
Eimeria xvaagioenaae Ortúzar-Ferreira & Berto n. sp.	*Patagioenas plumbea* (Vieillot, 1818)	South America, Brazil	Current work	Subspheroidal, ovoidal, or ellipsoidal	21–31 (26.1)	16–23 (20.1)	1.0–1.6 (1.31)	Smooth, 1.5–2.0 (1.8), sometimes with outer veil adhered	Absent	Randomly present at the longitudinal end or lateral, possibly with a micropyle cap	Present, 1–2	Elongate ellipsoidal to fusiform	13–18 (15.0)	6–8 (7.4)	1.8–2.3 (2.04)	Present, flattened to knob-like	Present, rounded to trapezoidal	Present, scattered

OQ790143 (COIBF1, ovoidal oocyst with micropyle cap and micropyle); OQ790144 (COIBF1, subspheroidal oocyst with micropyle); OQ790137 (COIBF1, ellipsoidal oocyst with micropyle cap, micropyle, and lateral micropyle); OQ790145 (MACOIII, subspheroidal oocyst with micropyle); OQ790140 (MACOIII, ellipsoidal oocyst with outer veil/rough wall, micropyle cap, micropyle, and lateral micropyle); OQ790139 (MACOIII, ellipsoidal oocyst with micropyle cap, micropyle, and lateral micropyle); OQ790138 (MAVCOXI, ellipsoidal oocyst with micropyle cap, micropyle, and lateral micropyle); OQ790142 (MAVCOXI, ellipsoidal oocyst with outer veil/rough wall, micropyle cap, micropyle, and lateral micropyle); and OQ790141 (MARI, ellipsoidal oocyst with outer veil/rough wall, micropyle cap, micropyle, and lateral micropyle).

ZooBank registration: to comply with the regulations set out in Article 8.5 of the amended 2012 version of the International Code of Zoological Nomenclature (ICZN 2012), details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) of the article is urn:lsid:zoobank.org:pub:6EECB8CA-B2FB-4732-864A-B83ECED5D32E. The LSID for the new name *Eimeria patagioenasae* Ortúzar-Ferreira & Berto n. sp. is urn:lsid:zoobank.org:act:F79F3CB8-22DD-4EE7-9F1E-8DDA8583A55F.

Site of infection: Unknown, oocysts were recovered from feces.

Prevalence: 67% (2 out of 3 birds examined).

Etymology: The specific name is derived from the generic name of the type host.

Statistical morphometric analyses

Means comparison analysis by ANOVA was performed according to the characteristic features in the oocyst wall, which were randomly present or absent. As shown in Table 3, means were equivalent ($p > 0.01$), in all morphometric dimensions between oocysts with and without the outer veil/rough wall, micropyle cap, micropyle, and lateral micropyle.

Histograms of length, width, and L/W ratio of the oocysts (Fig. 5) and sporocysts (Fig. 6) were regular as the frequencies increased and decreased in the classes of measures. These results indicate a single species/population in the measured oocysts and sporocysts, according to the guidelines of Sampaio (2002) and Berto et al. (2014).

Linear regression was performed for all oocysts (Fig. 7) and sporocysts (Fig. 8), but each oocyst shape related to combinations of characteristic features (morphotype) was individualized and identified on the graphs. As noted, there was no combination of characteristic features correlated to oocyst size/shape in both oocyst and sporocyst linear regressions, since the same combinations of characteristic features coincided in subspheroidal, ovoid, and ellipsoidal oocysts of different sizes. The very low R^2 value and the data points/oocysts distant from the regression line indicated the extremely polymorphic nature of the oocysts. In contrast, the linear regression of the sporocysts resulted in an R^2 value of 0.5, which is the borderline for determining a uniform distribution (Berto et al. 2014), as also confirmed by the closeness of the data points/sporocysts to the regression line.

Molecular and phylogenetic analyses

Molecular analyses of individual oocysts recovered from *P. plumbea* with different combinations of taxonomic characters (morphotypes) revealed the presence of eimeriid sequences differing from other coccidians deposited in the GenBank database.

At the COIBF1 *locus*, oocysts of three distinct morphotypes (subspheroidal with micropyle; ovoidal with micropyle cap and micropyle; and ellipsoidal with micropyle cap, micropyle, and lateral micropyle) were 100% identical to each other and 99% similar to *Isospora* spp. from passerines. *Eimeria columbinae* Ortúzar-Ferreira & Berto, 2019, which was described from ruddy ground doves *Columbina talpacoti* (Temminck, 1809) in southeastern Brazil, was the closest eimerian species with 98.2% similarity. *Eimeria labbeana* (Labbe, 1896) Pinto 1928 and *E. ferreirai*, which are two other *Eimeria* spp. recorded from Columbiformes sequenced to this same COIBF1 *locus*, had 93.5% and 91.6% similarity, respectively.

At the MACOIII *locus*, an ellipsoidal oocyst with an outer veil/rough wall, micropyle cap, micropyle, and lateral micropyle had 99.1% similarity with an oocyst of the same morphotype, except for the presence of the outer veil/rough wall. In contrast, this same ellipsoidal oocyst with an outer veil/rough wall, micropyle cap, micropyle, and lateral micropyle had 99.8% similarity to a subspheroidal oocyst with only a micropyle at the longitudinal end. From these oocysts, the closest sequences deposited in GenBank at this MACOIII *locus* were from *Eimeria dispersa* Tyzzer, 1929 and *Eimeria innocua* Moore & Brown, 1952, which are parasites of turkeys, along with some *Isospora* spp. from passerines, with a similarity of 93%.

At the MAVCOXI *locus*, the same previously mentioned ellipsoidal oocysts of the same morphotype, except for the outer veil/rough wall, were 100% identical to each other. In comparison with sequences deposited in the GenBank, the closest was *E. innocua* and *E. dispersa*, with 92.6% and 91.8% similarity, respectively.

At the MARI *locus*, only the ellipsoidal oocysts with outer veil/rough wall, micropyle cap, micropyle, and lateral micropyle were successful in amplification and sequencing. In comparison with sequences from GenBank, again,

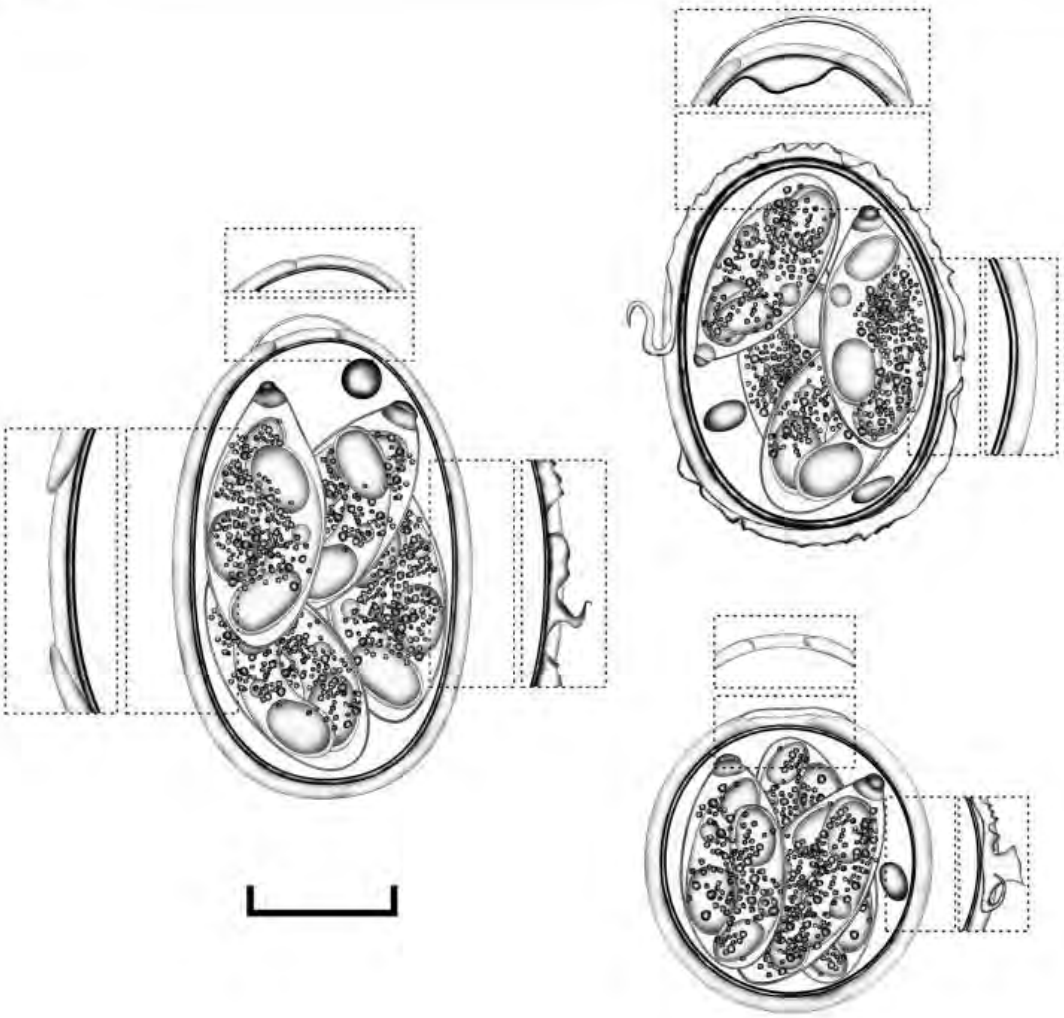

Fig. 2 Line drawings of ellipsoidal, ovoidal, and subspheroidal oocysts of *Eimeria patagioenasae* from plumbeous pigeons *Patagioenas plumbea*, with the random presence/absence of characteristic features associated with the oocyst wall, such as micropyle, micropyle cap, lateral micropyle, and outer veil/rough wall. Scale bar: 10 µm

E. innocua and *E. dispersa* were the closest, with 92.9% similarity.

Phylogenetic analysis based on the COIBF1, MACOIII, MAVCOXI, and MARI *loci* included sequences from coccidians available in GenBank (Figs. 9–12). An unnamed *Choleoeimeria* sp. (KT203395) from a prairie kingsnake, *Lampropeltis calligaster* (Harlan, 1827), was used as the outgroup. In the phylogenetic tree built from the COIBF1 *locus* (Fig. 9), the polymorphic oocysts from *P. plumbea* sat in a monophyletic group with *Isospora* spp. from passerines, being in polyphyly with the other *Eimeria* spp. of Columbiformes. In the phylogenetic tree built from the MACOIII *locus* (Fig. 10), the sequences of the current work sat in a large clade with *Eimeria* spp. from chickens, turkeys, geese, rabbits, and horses, in addition to *Isospora* spp. from passerines and other eimeriid coccidians. Finally, in the phylogenetic trees built from the MAVCOXI and MARI *loci* (Figs. 11 and 12), monophyletic groups with *E. innocua* and *E. dispersa* were formed. This relationship was reasonably supported in all analyses.

Discussion

The minimum sample number of host specimens aiming at descriptions or reports of coccidian species is not consensual or predetermined, as can be seen in the scientific literature, where new coccidian species were described even by a single host bird (Berto et al. 2011). On the other hand, the greater the number of samples (from different specimens, species,

Fig. 3 Photomicrographs of sporulated oocysts of *Eimeria patagioenasae* from plumbeous pigeons *Patagioenas plumbea*: ellipsoidal (**A–H**), ovoidal (**I–N**), and subspheroidal oocysts (**O–R**). Note the inner layer (il), smooth outer layer (sol), and/or outer veil/rough outer layer (ov/rol) in the oocyst wall, lateral micropyle (lm), micropyle (m), micropyle cap (mc), nucleus (n), polar granule (pg), refractile body (rb), sporocyst residuum (sr), Stieda (sb), and sub-stieda (ssb) bodies. Scale bar: 10 µm

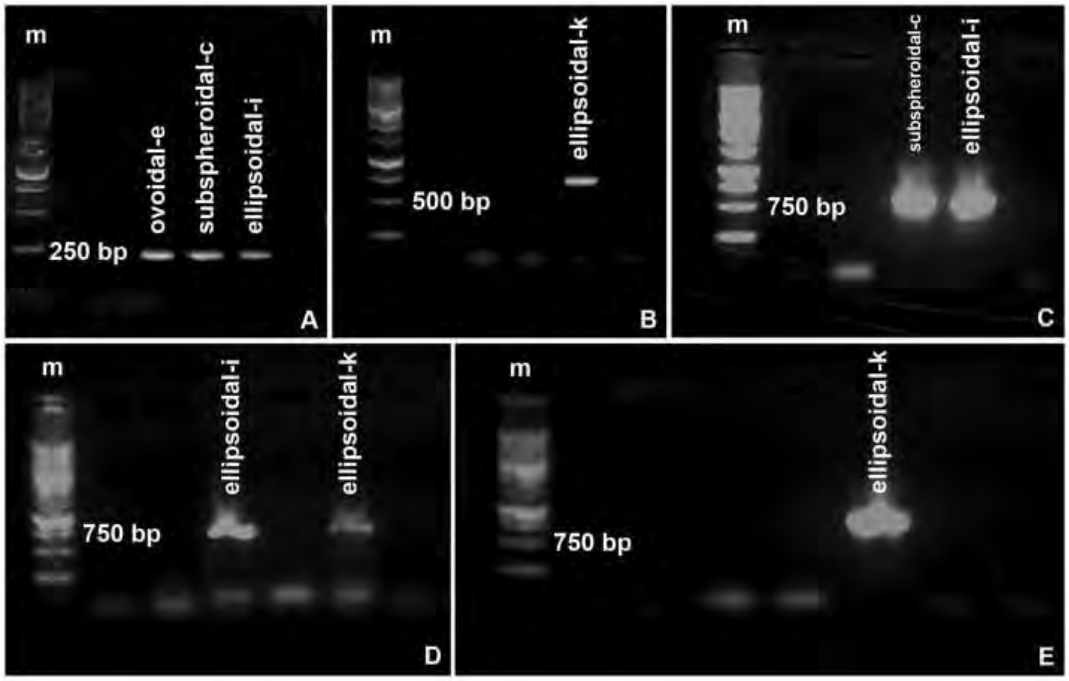

Fig. 4 Electrophoretic profile of PCR for COIBF1 (**A**), MACOIII (**B**, **C**), MAVCOXI (**D**), and MARI (**E**) *loci* of the mitochondrial genome of *Eimeria patagioenasae* from plumbeous pigeons *Patagioenas plumbea*, obtained in 1.2% agarose gel. Molecular weight marker (m) Promega 1 kb DNA ladder (250–10,000 bp). Morphotypes "subspheroidal-c", "ovoidal-e", "ellipsoidal-i" and "ellipsoidal-k" refer to the extraction of DNA from individual oocysts subspheroidal with micropyle; ovoidal with micropyle cap and micropyle; ellipsoidal with micropyle cap, micropyle, and lateral micropyle; and ellipsoidal with outer veil/rough wall, micropyle cap, micropyle, and lateral micropyle; respectively

Table 3 Statistical analysis of mean comparisons of oocysts and sporocysts related to taxonomic characters randomly present or absent in oocysts of *Eimeria patagioenasae*

Morphometric dimensions	Characteristic features							
	Outer veil/rough wall		Micropyle cap		Micropyle		Lateral micropyle	
	Present ($n=15$)	Absent ($n=25$)	Present ($n=19$)	Absent ($n=21$)	Present ($n=31$)	Absent ($n=9$)	Present ($n=14$)	Absent ($n=6$)
Oocyst								
Length (µm)	26.0 (22–30)[a]	26.2 (21–31)[a]	26.9 (23–31)[a]	25.5 (21–31)[a]	26.1 (21–31)[a]	26.2 (22–31)[a]	26.6 (23–31)[a]	25.9 (21–31)[a]
Width (µm)	20.0 (18–22)[a]	20.1 (16–23)[a]	19.7 (16–22)[a]	20.4 (18–23)[a]	20.0 (16–22)[a]	20.4 (19–23)[a]	20.0 (16–22)[a]	20.1 (16–23)[a]
Length/width ratio	1.3 (1.1–1.5)[a]	1.3 (1.0–1.6)[a]	1.4 (1.1–1.6)[a]	1.3 (1.0–1.5)[a]	1.3 (1.0–1.6)[a]	1.3 (1.1–1.5)[a]	1.3 (1.2–1.5)[a]	1.3 (1.0–1.6)[a]
Sporocyst								
Length (µm)	15.6 (15–17)[a]	14.7 (13–18)[a]	15.1 (13–18)[a]	15.0 (13–17)[a]	14.9 (13–18)[a]	15.4 (15–17)[a]	15.7 (14–17)[a]	14.8 (13–18)[a]
Width (µm)	7.6 (6–8)[a]	7.2 (7–8)[a]	7.4 (7–8)[a]	7.3 (6–8)[a]	7.3 (7–8)[a]	7.4 (6–8)[a]	7.7 (7–8)[a]	7.2 (6–8)[a]
Length/width ratio	2.1 (1.9–2.3)[a]	2.0 (1.8–2.3)[a]	2.0 (1.8–2.3)[a]	2.1 (1.9–2.3)[a]	2.0 (1.8–2.3)[a]	2.1 (1.9–2.3)[a]	2.0 (1.9–2.2)[a]	2.0 (1.8–2.3)[a]

[a]All means (same letters) within each taxonomic character column were equivalent ($p > 0.01$)

and localities), the greater the delimitation and accuracy of the standardization/typification of a coccidian species (Ortúzar-Ferreira et al. 2021; Berto and Lopes 2020). The low number of plumbeous pigeons (*P. plumbea*) captured and analyzed in the current study is justified by the rarity and difficulty of capturing this pigeon in the Itatiaia National

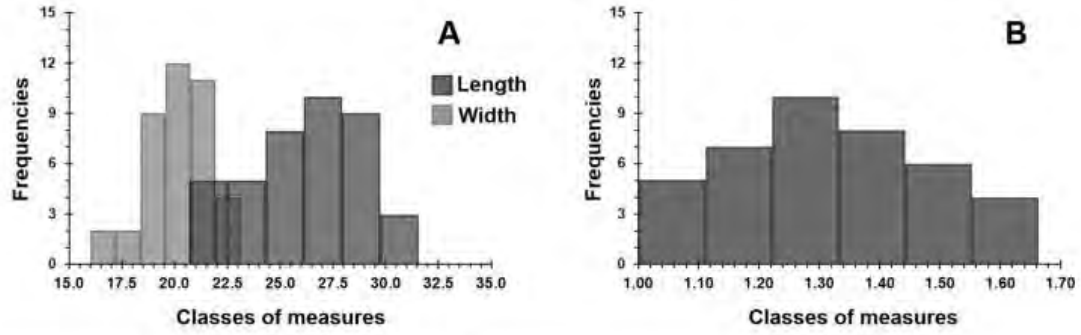

Fig. 5 Histograms of length and width (**A**) and length/width ratio (**B**) of oocysts of *Eimeria patagioenasae* from plumbeous pigeons *Patagioenas plumbea*

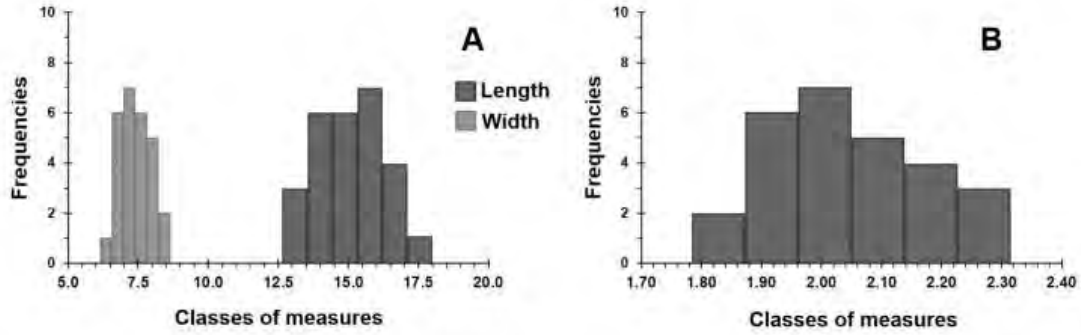

Fig. 6 Histograms of length and width (**A**) and length/width ratio (**B**) of sporocysts in oocysts of *Eimeria patagioenasae* from plumbeous pigeons *Patagioenas plumbea*

Park. After more than 10 years of bimonthly expeditions in the park, only these three specimens of *P. plumbea* were captured in this area of very difficult access and fieldwork in the high-altitude plateau of the Itatiaia National Park. In any case, the very reasonable number of oocysts analyzed (40) from two of the three captured plumbeous pigeons ensured an accurate morphological, statistical, and molecular study, not least because the complementary molecular studies were carried out on individual oocysts.

The oocysts, identified and described as *E. patagioenasae*, were extremely variable in size, shape, and the presence and absence of various characteristic features associated with the oocyst wall (Figs. 2 and 3). These variations were largely random; that is, it was not possible to standardize/typify certain sizes and shapes associated with the presence/absence of certain characteristic features (morphotypes). This assertion is also supported by the linear regression of the oocysts (Fig. 7), where 11 different combinations of characteristic features were randomly distributed along the regression line into oocysts of different sizes and shapes. If each combination of characteristic features associated with a shape/size of oocyst were overestimated, 19 different morphotypes/species could be identified/described. In contrast, sporocysts were uniform, morphologically and morphometrically, within oocysts of distinct morphotypes, as shown in Fig. 8, reinforcing the hypothesis of a single species in the observed oocysts. Allied to this, the histograms were regular in the frequencies distributed in the classes of measures (Figs. 5 and 6), and the means comparison analysis showed that there are no significant differences between oocysts with and without micropyle, micropyle cap, lateral micropyle, and outer veil/rough wall (Table 3); therefore, strongly indicating the presence of a single species in the fecal samples of *P. plumbea*. Some eimerian species were differentiated and described based on moderate variations in size, shape, and roughness of the oocyst wall, as in Casas et al. (1995) in the description of three new species of *Eimeria* spp. from capybaras, in contrast to Flausino et al. (2014), which redescribed *Eimeria caviae* Sheather, 1924, as having subspheroidal, ovoidal, and ellipsoidal oocysts from Guinea pigs. In the current work, based primarily on morphological and statistical morphometric analyses of the oocysts, a conservative evaluation and identification were defined, indicating the extreme variations in certain taxonomic characters as intraspecific, that is, polymorphism.

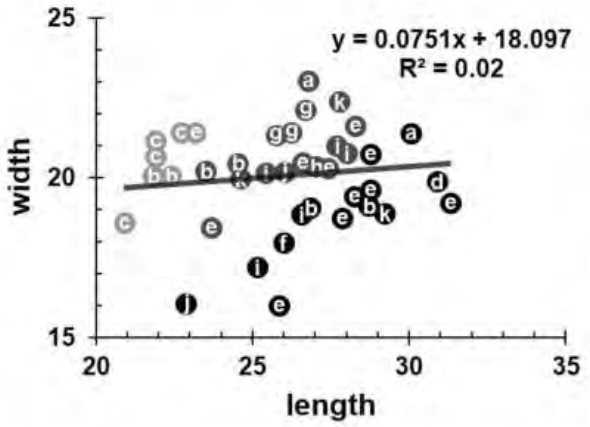

Fig. 7 Distribution of subspheroidal (light grey), ovoidal (dark grey), and ellipsoidal (black) oocysts of *Eimeria patagioenasae* in linear regression ($n=40$). The letters indicate the following combinations of characteristic features (morphotypes): a, oocyst wall smooth and without associated morphological structures; b, with outer veil/rough wall; c, with micropyle; d, with lateral micropyle; e, with micropyle cap and micropyle; f, with outer veil/rough wall and micropyle; g, with micropyle and lateral micropyle; h, with outer veil/rough wall, micropyle cap, micropyle; i, with outer veil/rough wall, micropyle and lateral micropyle; j, with micropyle cap, micropyle and lateral micropyle; and k, with outer veil/rough wall, micropyle cap, micropyle and lateral micropyle

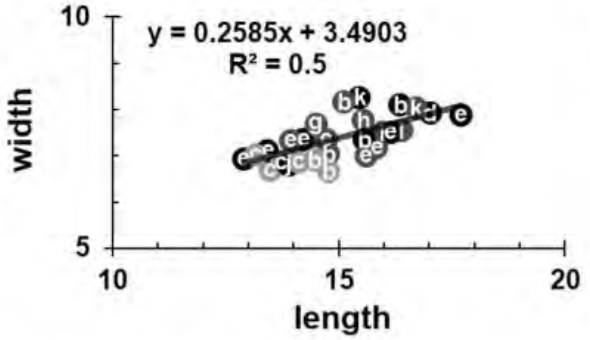

Fig. 8 Distribution of sporocysts in subspheroidal (light grey), ovoidal (dark grey), and ellipsoidal (black) oocysts of *Eimeria patagioenasae* in linear regression ($n=27$). The letters indicate the following combinations of characteristic features (morphotypes): b, with outer veil/rough wall; c, with micropyle; d, with lateral micropyle; e, with micropyle cap and micropyle; g, with micropyle and lateral micropyle; h, with outer veil/rough wall, micropyle cap, and micropyle; i, with outer veil/rough wall, micropyle, and lateral micropyle; j, with micropyle cap, micropyle, and lateral micropyle; k, with outer veil/rough wall, micropyle cap, micropyle, and lateral micropyle

Polymorphism is a population characteristic in which a single species has variations in its shape, size, and other characteristic features; therefore, a variation at the species level (intraspecific) (Gardner and Duszynski 1990; Wiens 1999; Amorim 2002; Berto and Lopes 2020). In population genetics, the term polymorphism indicates a transience of alleles where two or more alleles from the same *locus* coexist; that is, polymorphism is the phenotypic/genotypic variations intrinsic to a species (Amorim 2002). However, in addition to this intrinsic polymorphism of the species, other factors may be associated. From the reviews by Fayer (1980) to Berto and Lopes (2020), several authors indicate that stress, nutrition and immunity of the host, phenotypic plasticity, infective dose, time of oocyst shedding in the patent period, anticoccidial drugs, abiotic factors associated with sporulation, and measurement methodology may be related to oocyst polymorphism. In this context, the following works are noteworthy: Duszynski (1971) reports an increase in the size of oocysts of *Eimeria separata* Becker & Hall, 1931, during the patent period; Parker and Duszynski (1986) identified an extreme polymorphism in oocysts of *Eimeria reichenowi* Yakimoff & Matschoulsky, 1935, later related to a phenotypic plasticity (Gardner and Duszynski 1990), which is defined as the ability of a single genotype to exhibit a range of phenotypes in response to variation in the environment (Pigliucci 2001); Fayer (1980) and Berto and Lopes (2020) synthesize that the different wild, anthropized and confinement environments of the hosts can interfere with the morphometry and the number of oocysts shedding, which are reported by Gomez et al. (1982) and Berto et al. (2008); Greif et al. (1996) found that resistance to anticoccidial drugs can generate polymorphism; and, finally, Berto et al. (2014) demonstrate that in coccidian species with oocysts with an L/W ratio greater than 1.1 (generally ovoidal/ellipsoidal shape), the observation of polymorphism is more common since the dimensional measurement of the oocyst (which is three-dimensional) is inaccurate when the oocysts are not in strictly longitudinal position, while in subspheroidal oocysts (L/W ratio of 1.0–1.1), this inaccuracy is greatly attenuated. In the context of the latter case, all ovoidal and ellipsoidal oocysts measured in the current work were carefully checked and measured in the longitudinal position, reducing this morphometric inaccuracy as much as possible.

From an evolutionary point of view, polymorphism may represent an ongoing speciation process, which Wiens (1999) names macroevolution, that is, the gradual process in which certain taxonomic characters can be eliminated or incorporated in the formation of a new species. Wiens (1999) also asserts that polymorphism can have a profound impact on species delimitation and, consequently, on taxonomic and phylogenetic studies; therefore, polymorphic characters cannot be neglected. Some studies report this polymorphic process of speciation in eimeriid coccidians: Gardner and Duszynski (1990) observed polymorphism in oocysts of *Eimeria opimi* Lambert, Gardner & Duszynski, 1988 in different species of tuco-tucos *Ctenomys* spp. in Bolivia, which was associated with a process of speciation

Fig. 9 Phylogenetic relationship of *Eimeria patagioenasae* from plumbeous pigeons *Patagioenas plumbea* inferred by Bayesian analysis for a *locus* (COIBF1) within *cox1* gene of the mitochondrial genome. Numbers at the nodes show bootstrap values derived from Maximum likelihood analysis/posterior probabilities under the Bayesian Inference analysis. Only bootstrap supports and posterior probabilities higher than 50% or 0.50, respectively, are displayed. Morphotypes "subspheroidal-c", "ovoidal-e" and "ellipsoidal-i" refer to oocysts subspheroidal with micropyle; ovoidal with micropyle cap and micropyle; and ellipsoidal with micropyle cap, micropyle, and lateral micropyle; respectively

Fig. 10 Phylogenetic relationship of *Eimeria patagioenasae* from plumbeous pigeons *Patagioenas plumbea* inferred by Bayesian analysis for a *locus* (MACOIII) that partially covers the *cox3* gene of the mitochondrial genome. Numbers at the nodes show bootstrap values derived from maximum likelihood analysis/posterior probabilities under the Bayesian Inference analysis. Only bootstrap supports and posterior probabilities higher than 50% or 0.50, respectively, are displayed. Morphotypes "subspheroidal-c", "ellipsoidal-k" and "ellipsoidal-i" refer to oocysts subspheroidal with micropyle; ellipsoidal with outer veil/rough wall, micropyle cap, micropyle, and lateral micropyle; and ellipsoidal with micropyle cap, micropyle, and lateral micropyle; respectively

and host adaptation; Silva-Carvalho et al. (2020) observed morphological and genotypic variations in *Isospora parnaitatiaiensis* Silva, Rodrigues, Lopes, Berto, Luz, Ferreira & Lopes, 2015 from different antbird species, which were also associated with the process of adaptive speciation to different host species. In this context, Berto and Lopes (2020) summarize that the intense genetic recombinations at certain moments of speciation, especially in adapting

Fig. 11 Phylogenetic relationship of *Eimeria patagioenasae* from plumbeous pigeons *Patagioenas plumbea* inferred by Bayesian analysis for a *locus* (MAVCOXI) within *cox1* gene of the mitochondrial genome. Numbers at the nodes show bootstrap values derived from maximum likelihood analysis/posterior probabilities under the Bayesian Inference analysis. Only bootstrap supports and posterior probabilities higher than 50% or 0.50, respectively, are displayed. Morphotypes "ellipsoidal-k" and "ellipsoidal-i" refer to oocysts ellipsoidal with outer veil/rough wall, micropyle cap, micropyle, and lateral micropyle; and ellipsoidal with micropyle cap, micropyle, and lateral micropyle; respectively

Fig. 12 Phylogenetic relationship of *Eimeria patagioenasae* from plumbeous pigeons *Patagioenas plumbea* inferred by Bayesian analysis for a *locus* (MARI) which occupies fragments of small and large subunits rDNA of the mitochondrial genome. Numbers at the nodes show bootstrap values derived from maximum likelihood analysis/posterior probabilities under the Bayesian Inference analysis. Only bootstrap supports and posterior probabilities higher than 50% or 0.50, respectively, are displayed. Morphotype "ellipsoidal-k" refers to an oocyst ellipsoidal with an outer veil/rough wall, micropyle cap, micropyle, and lateral micropyle

to new hosts, should generate random taxonomic characters depending on whether the genes are present/active or not. Therefore, the possibility that *E. patagioenasae* is in a polymorphic process of speciation, and host adaptation is prominent. The oocysts of *E. patagioenasae* share some typical taxonomic characteristics with *E. ferreirai*, such as the size and micropyle in the longitudinal position of the oocyst, in addition to *E. labbeana*, which has longitudinal and lateral micropyles. Furthermore, *E. ferreirai* has the same type locality as *E. patagioenasae*, having its hosts sympatric in the Itatiaia National Park in southeastern Brazil. Possibly *E. patagioenasae* is speciating in an adaptation to *P. plumbea* from an ancestral eimerian common to *Eimeria* spp. of Columbiformes, in a process where many

genetic recombinations occur leading to the observed phenotypic polymorphism.

The molecular results confirmed the definition of a single species in the oocysts (of different morphotypes) shed by the plumbeous pigeons *P. plumbea*, since small genetic divergences were observed only in the MACOIII *locus*, while for the COIBF1 and MAVCOXII *loci*, the sequences were identical between the different morphotypes. It is noteworthy that the highest molecular divergence of 0.9% (5 nt; 561/566 identities) at the MACOIII *locus* was between oocysts morphologically similar (ellipsoid oocysts with more than three characteristic features associated with the oocyst wall), compared to a molecular divergence of 0.2% (1 nt; 565/566 identities) between the oocysts most morphologically dissimilar (subsespheroidal and ellipsoidal oocysts largely distinct in the oocyst wall). As such, it is understood that the small molecular divergences detected at the MACOIII *locus* were not associated with the different morphotypes, reinforcing the decision of the current work to identify the different morphotypes as intraspecific variations of *E. patagioenasae*. At the same time, the nucleotide substitutions at the MACOIII *locus* show some genetic polymorphism, which, despite not having been directly correlated with specific morphotypes, emphasizes that the morphological and morphometric polymorphism in the oocysts must be intrinsic to the species or related to the process of speciation/host adaptation and not related to the previously mentioned external factors.

In contrast to current work, Hafeez et al. (2014) named two *Isospora* spp., morphologically and morphometrically undifferentiated, based on a molecular divergence of 1.4% (11 nt) at a 761-nt *locus* of the *cox1* gene. The molecular divergence obtained by Hafeez et al. (2014) was reasonably close to that of the current study (1.4% vs. 0.9%), but it is important to emphasize that these are distinct and non-overlapping *loci* in mitochondrial DNA. The *locus* used in Hafeez et al. (2014) was positioned between ~ 1700 and 2500 nt of mitochondrial DNA, which partially overlaps the COIBF1 and MAVCOXII *loci*, whereby 100% identity was obtained between the morphotypes of the current work (Fig. 1).

There are only five sequences of *loci* of the *cox1* gene from *Eimeria* spp. of Columbiformes deposited in GenBank. The first deposits were from Yang et al. (2016) for *loci* of the small (18S) and large (28S) subunit ribosomal RNA and *cox1* genes for an *Eimeria* sp. recovered from *Columba livia* Gmelin, 1789, in Australia. *Eimeria columbinae* was sequenced for the COIBF1 *locus* and a *locus* of the 18S gene (Ortúzar-Ferreira et al. 2020). *Eimeria ferreirai* and *E. chalcoptereae* were described with deposits of sequences in the *cox1* gene and also 18 s and 28 s genes for *E. chalcoptereae* (Yang et al. 2020; Oliveira et al. 2020). *Eimeria labbeana* was morphologically supplemented and had sequences from the COIBF1 *locus* and a *locus* of 18S deposited (Oliveira et al. 2021). Finally, Taroda et al. (2020) deposited sequences of a *locus* at the *cox1* gene from eimerian oocysts of eared doves *Zenaida auriculata* (Des Murs, 1847) in Brazil, but no species were morphologically identified in this work. Thus, *E. patagioenasae* becomes the fifth nominal species recorded from Columbiformes to have a molecular identification.

Molecular analyses of *loci* of the 18S and 28S nuclear genes were intended in the current study, but the amplification was not successful, possibly due to the DNA extraction methodology from an individual oocyst that provides few copies of nuclear DNA, unlike the greater amount of mitochondrial DNA available in each sporozoite/oocyst (Dolnik et al. 2009). Anyway, the use of *loci* of the 18S and 28S genes is more suitable for the study of deeper evolutionary events, whereas the mitochondrial genes have been considered the most suitable for species differentiation and detection of recent evolutionary events (Ogedengbe et al. 2011, 2015); therefore, they are more consistent with the objectives of the current study.

The phylogenetic analyses were inconclusive because they did not form monophyletic groups with *Eimeria* spp. from Columbiformes, which would be an expected result for the COIBF1 *locus*, or because there are no sequences of eimerians from Columbiformes deposited for the same genic regions of the MACOIII, MAVCOXI, and MARI *loci*. In fact, the COIBF1 *locus*, despite being the most successful in amplification from an individual oocyst, has been shown to be unsuitable for phylogenetic analysis because it does not show results consistent with phylogenetic, morphological, or ecological hypotheses (Ortúzar-Ferreira et al. 2022). On the other hand, the MACOIII, MAVCOXI, and MARI *loci*, which have longer sequences and are in other regions of the mitochondrial DNA (Fig. 1), showed monophyletic groups associated with their respective host groups (Figs. 10, 11 and 12), proving to be *loci* with a promising future in phylogenetic studies when more eimerian coccidians are sequenced in these same genic regions. It is worth highlighting the MAVCOXI *locus*, which showed consistent and well-supported results in the phylogenetic tree (Fig. 11) and belongs to the *cox1* gene, which is considered one of the main genes for species delimitation in the so-called "barcode of life" (Ogedengbe et al. 2011).

In conclusion, the delimitation and description of species must be a detailed and multifactorial process, mainly in the case of extremely polymorphic species; in order to avoid misidentifications and invalid descriptions, at the same time it must present with greater comprehensibility and specificities the characteristics inherent to a species (Dayrat 2005). In this context, "Integrative Taxonomy" is the name given to the use of multiple complementary tools that allow analyzing and inferring about a biological material to describe biodiversity (Dayrat 2005; Fujita et al. 2012; Hoberg et al.

2015). In the case of the identification of eimeriid coccidian species, the multifactorial approach of the analysis of morphology, morphometric statistics, molecular and phylogenetic studies, and considerations on the biology and ecology of the parasite and the host (e.g., specificity, sites of infection, and ecological niches) express the Integrative Taxonomy (Dayrat 2005; Berto et al. 2014; 2023; Berto and Lopes 2020). Thus, the multifactorial approach of the current work supported the definition of the polymorphic oocysts shed from *P. plumbea* as a single species, *E. patagioenasae*, making this the twenty-second eimerian description from Columbiformes.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00436-023-08045-5.

Acknowledgements We are thankful to the staff at the Parque Nacional do Itatiaia, mainly to the research coordinators Dr Léo Nascimento (previous) and Marcelo Souza Motta (current), who allowed us to access and use some facilities during the expeditions.

Author contribution The study was designed by MSO, VML and BPB. Field work was performed by CNO-F, LASA and BPB. Laboratory procedures for maintenance, recovery, measurements, photomicrographs and isolation of oocysts were performed by CNO-F and LASA. Primers for amplification of new *loci* were designed by MSO and VML. DNA extraction, amplification and sequencing were performed by MSO, ERM and VML. New sequences were deposited in GenBank by VML. Morphometric statistical and phylogenetic analyses, figure editing and line drawings were performed by BPB. The manuscript was written by CNO-F, MSO and BPB and subsequently revised by all other authors. All authors read and approved the final manuscript.

Funding This study was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ). CNO-F and LASA have scholarships from CAPES (Grant/Award Number: 001). MSO has a postdoctoral scholarship from FAPERJ (Grant/Award Number: E-26/204.228/2021). VML has a fellowship from FAPERJ (Grant/Award Number: E-27/211.566/2021). BPB has a fellowship from CNPq (Grant/Award Number: 302345/2022–1) and from FAPERJ (Grant/Award Number: E-26/200.565/2023).

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval Field-collecting permits were issued by the Chico Mendes Institute for Biodiversity Conservation (Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio)), through the Biodiversity Authorization and Information System (Sistema de Autorização e Informação em Biodiversidade (SISBIO)) under license number 70132, and the Animal Ethics Committee (Comitê de Ética no Uso de Animais (CEUA)) of the University of Grande Rio (Universidade do Grande Rio (UNIGRANRIO)) under protocol number 021/2019. Banding permits and metal rings were issued by CEMAVE/ICMBio (Senior Ringer: BPB, registration 5967850). All applicable institutional, national, and international guidelines for the care and use of animals were followed.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

Adriano EA, Thyssen PJ, Cordeiro NS (2000) *Eimeria curvata* n. sp. (Apicomplexa: Eimeriidae) in *Columbina talpacoti* and *Scardafella squammata* (Aves: Columbidae) from Brazil. Mem Inst Oswaldo Cruz 95:53–55. https://doi.org/10.1590/S0074-02762000000100008

Adriano EA, Thyssen PJ, Cordeiro NS (2003) A new species of *Eimeria* from the eared dove *Zenaida auriculata* (Aves: Columbidae) in Brazil. Acta Protozool 42:71–73

Alyousif MS, Al-Shawa YR, Al-Asiri SS (2009) *Eimeria livialis* sp. n. (Apicomplexa: Eimeriidae) from the domestic pigeon, *Columba livia* domestica in Saudi Arabia. J Egypt Soc Parasitol 39:383–388

Amorim DS (2002) Fundamentos de Sistemática Filogenética. Holos Editora, Ribeirão Preto

Atkinson CT, Thomas NJ, Hunter DB (2008) Parasitic diseases of wild birds. Wiley-Blackwell, Singapore

Ayres M, Ayres M, Ayres DL, Santos AAS (2007) BioStat aplicações estatísticas nas áreas de Ciências Biomédicas. Mamirauá, Belém

Ball SJ, Daszak P, Swinnerton KR, Jones CG, Snow KR (2012) A new species of *Eimeria* (Apicomplexa:Eimeriidae) from the endangered pink pigeon, *Nesoenas mayeri* (Prévost, 1843) Cheke, 2005 (Columbiformes) in Mauritius. Afr Zool 47:369–372

Bandyopadhyay PK, Bhakta JN, Shukla R (2006) A new *Eimeria* species (Protozoa: Apicomplexa: Sporozoea) from the blue rock pigeon *Columba livia* (Aves: Columbidae). Zoos Print J 21:2386–2387

Berto BP, Lopes CWG (2020) Coccidia of wild birds as ecological biomarkers: some approaches on parasite-host-environment interaction. J Parasitol 106:707–713. https://doi.org/10.1645/19-148

Berto BP, Flausino W, Almeida CRR, Lopes CWG (2008) Polymorphism of *Tyzzeria parvula* (Kotlán, 1933) Klimes, 1963 (Apicomplexa: Eimeriidae) oocysts from the greylag geese, *Anser anser* L., 1758 from two distinct sites. Rev Bras Med Vet 30:215–219

Berto BP, Flausino W, McIntosh D, Teixeira-Filho WL, Lopes CWG (2011) Coccidia of New World passerine birds (Aves: Passeriformes): a review of *Eimeria* Schneider, 1875 and *Isospora* Schneider, 1881 (Apicomplexa: Eimeriidae). Syst Parasitol 80:159–204. https://doi.org/10.1007/s11230-011-9317-8

Berto BP, McIntosh D, Lopes CWG (2014) Studies on coccidian oocysts (Apicomplexa: Eucoccidiorida). Rev Bras Parasitol Vet 23:1–15. https://doi.org/10.1590/S1984-29612014001

Berto BP, Machado EL, Hossotani CMDS, Beretta BMS, Silva DRR, Nakamura AA, Meireles MV (2023) Integrative taxonomy for the traditional coccidians (Chromista: Miozoa: Eimeriidae) from island canaries (Aves: Passeriformes: Fringillidae): worldwide distribution, morphological and molecular characterization, revaluations and establishment of junior synonyms. Syst Parasitol 100:245–259. https://doi.org/10.1007/s11230-023-10084-6

BirdLife International (2023) Species factsheet: *Patagioenas plumbea*. http://www.birdlife.org. Accessed 14 April 2023

Casas MC, Duszynski DW, Zalles LM (1995) Three new eimerians in capybara (*Hydrochaeris hydrochaeris*) populations from Eastern Bolivia and Southern Venezuela. J Parasitol 81:247–251. https://doi.org/10.2307/3283928

Chatterjee DK, Ray HN (1969) *Eimeria kapotei* n. sp., from the domestic pigeon, *Columba livia intermedia*. Proc 24th Indian Sci Cong 56:512

Dayrat B (2005) Towards integrative taxonomy. Biol J Linn Soc 85:407–415. https://doi.org/10.1111/j.1095-8312.2005.00503.x

Dolnik O (2006) The relative stability of chronic *Isospora sylvianthina* (Protozoa: Apicomplexa) infection in blackcaps (*Sylvia atricapilla*): evaluation of a simplified method of estimating isosporan infection intensity in passerine birds. Parasitol Res 100:155–160. https://doi.org/10.1007/s00436-006-0253-5

Dolnik OV, Palinauskas V, Bensch S (2009) Individual oocysts of *Isospora* (Apicomplexa: Coccidia) parasites from avian feces: from photo to sequence. J Parasitol 95:169–174. https://doi.org/10.1645/GE-1873.1

Duszynski DW (1971) Increase in size of *Eimeria separata* oocysts during patency. J Parasitol 57:948–952. https://doi.org/10.2307/3277841

Duszynski DW (2021) Biodiversity of the Coccidia (Apicomplexa: Conoidasida) in vertebrates: what we know, what we do not know, and what needs to be done. Folia Parasitol 68:001. https://doi.org/10.14411/fp.2021.001

Duszynski DW, Wilber PG (1997) A guideline for the preparation of species descriptions in the Eimeriidae. J Parasitol 83:333–336. https://doi.org/10.2307/3284470

Fayer R (1980) Epidemiology of protozoan infections: the coccidia. Vet Parasitol 6:75–103. https://doi.org/10.1016/0304-4017(80)90039-4

Flausino G, Lopes CWG, Teixeira-Fillho WL, Furtado TT, Mcintosh D, Berto BP (2014) Phenotypic and genotypic characterization of *Eimeria caviae* from guinea pigs (*Cavia porcellus*). Acta Protozool 53:269–276. https://doi.org/10.4467/16890027AP.14.024.1999

Fujita MK, Leaché AD, Burbrink FT, McGuire JA, Moritz C (2012) Coalescent-based species delimitation in an integrative taxonomy. Trends Ecol Evol 27:480–488. https://doi.org/10.1016/j.tree.2012.04.012

Gardner SL, Duszynski DW (1990) Polymorphism of eimerian oocysts can be a problem in naturally infected hosts: an exemple from subterranean rodents in Bolivia. J Parasitology 76:805–811. https://doi.org/10.2307/3282798

Golemansky V (1976) Three new coccidian species (Coccidia: Eimeriidae) found in wild birds from Bulgaria. Acta Protozool 15:399–404

Gomez FM, Navarrete I, Rodriguez RL (1982) Influence of environmental factors on different populations of *Isospora lacazei* Labbe 1893 (Protozoa: Apicomplexa). Rev Iber Parasitol 42:185–196

Greif G, Stephan B, Haberkorn A (1996) Intraspecific polymorphisms of *Eimeria* species due to resistance against anticoccidial drugs. Parasitol Res 82:706–714. https://doi.org/10.1007/s004360050189

Hafeez MA, Stasiak I, Delnatte P, El-Sherry S, Smith DA, Barta JR (2014) Description of two new *Isospora* species causing visceral coccidiosis in captive superb glossy starlings, *Lamprotornis superbus* (Aves: Sturnidae). Parasitol Res 113:3287–3297. https://doi.org/10.1007/s00436-014-3992-8

Hoberg EP, Agosta SJ, Boeger WA, Brooks DR (2015) An integrated parasitology: revealing the elephant through tradition and invention. Trends Parasitol 31:128–133. https://doi.org/10.1016/j.pt.2014.11.005

ICMBIO (2023) Parque Nacional do Itatiaia. http://www.icmbio.gov.br/parnaitatiaia. Accessed 14 April 2023

ICZN (2012) International Commission on Zoological Nomenclature: Amendment of Articles 8, 9, 10, 21 and 78 of the International Code of Zoological Nomenclature to expand and refine methods of publication. Bull Zool Nomencl 69:161–169. https://doi.org/10.21805/bzn.v69i3.a8.161

Jamriška J, Modrý D (2012) A new species of *Eimeria* Schneider, 1875 (Apicomplexa: Eimeriidae) from the common wood pigeon *Columba palumbus* Linnaeus, 1758 (Aves: Columbidae). Acta Protozool 51:329–333. https://doi.org/10.4467/16890027AP.12.026.0786

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Bio Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096

McQuistion TE (1991) *Eimeria palumbi*, a new coccidian parasite (Apicomplexa: Eimeriidae) from the Galapagos dove (*Zenaida galapagoensis*). Trans Am Microsc Soc 110:178–181. https://doi.org/10.2307/3226755

Mello DJM, Mello GJM, Mallet-Rodrigues F, Lima LM (2020) Aves do Sudeste do Brasil: Guia de identificação. Editoração Irmãos Mello, Rio de Janeiro

Mitra AN, Das-Gupta M (1937) On a species of Eimeria (Coccidia–Sporozoa) from the intestine of a pigeon, Columba intermedia. Proc 24th Indian Sci Cong 24:291

Nieschulz O (1935) Ueber Kokzidien der Haustauben. Zentralbl Bakteriol Mikrobiol Hyg 134:390–393

Norton CC, Joyner LP (1981) *Eimeria acervulina* and *E. mivati*: oocysts, life-cycle and ability to develop in the chicken embryo. Parasitology 83:269–279. https://doi.org/10.1017/S0031182000085280

Ogedengbe JD, Hanner RH, Barta JR (2011) DNA barcoding identifies *Eimeria* species and contributes to the phylogenetics of coccidian parasites (Eimeriorina, Apicomplexa, Alveolata). Int J Parasitol 41:843–850. https://doi.org/10.1016/j.ijpara.2011.03.007

Ogedengbe JD, Ogedengbe ME, Hafeez MA, Barta JR (2015) Molecular phylogenetics of eimeriid coccidia (Eimeriidae, Eimeriorina, Apicomplexa, Alveolata): a preliminary multi-gene and multi-genome approach. Parasitol Res 114:4149–4160. https://doi.org/10.1007/s00436-015-4646-1

Oliveira MS, Genovez-Oliveira JL, Ortúzar-Ferreira CN, Maronezi C, Thode-Filho S, Cardozo SV, Oliveira AA, Lima VM, Berto BP (2020) *Eimeria ferreirai* n. sp. (Chromista: Miozoa: Eimeriidae) from doves *Leptotila* spp. (Columbiformes: Columbidae) from Brazil. Zootaxa 4821:148–160. https://doi.org/10.11646/zootaxa.4821.1.8

Oliveira MS, Ramilo DW, Mello ER, Cardozo SV, Caetano I, Brazio E, Fonseca IP, Oliveira AA, Lima VM, Berto BP (2021) Supplementary morphological data and molecular analyses of *Eimeria labbeana* (Labbé, 1896) Pinto, 1928 (Chromista: Miozoa: Eimeriidae) from columbiform birds in Portugal. Parasitol Res 120:3569–3580. https://doi.org/10.1007/s00436-021-07300-x

Ortúzar-Ferreira CN, Oliveira MS, Genovez-Oliveira JL, Franco HA, Thode-Filho S, Oliveira AA, Lima VM, Ferreira I, Berto BP (2020) Coccidia of Columbiformes of the world: a taxonomic review of its Eimeriidae species and *Eimeria columbinae* n. sp. from *Columbina talpacoti* (Temminck, 1809) from Brazil. Parasitol Res 119:329–333. https://doi.org/10.1007/s00436-019-06514-4

Ortúzar-Ferreira CN, Mello ER, Melo JO, Oliveira MS, Thode-Filho S, Cardozo SV, Oliveira ÁA, Lima VM, Ferreira I, Berto BP (2021) Redescription and molecular identification of *Isospora feroxis* Berto, Luz, Flausino, Ferreira & Lopes, 2009 (Eimeriidae) from tyrant-flycatchers (Tyrannoidea) in South America. Syst Parasitol 98:333–341. https://doi.org/10.1007/s11230-021-09981-5

Ortúzar-Ferreira CN, Andrade LAS, Genovez-Oliveira JL, Oliveira MS, Mello ER, Cardozo SV, Oliveira ÁA, Lima VM, Ferreira I, Berto BP (2022) Molecular identification of *Isospora coerebae* Berto, Flausino, Luz, Ferreira & Lopes, 2010 (Chromista: Miozoa: Eimeriidae) from the bananaquit *Coereba flaveola* (Linnaeus, 1758) (Passeriformes: Thraupidae: Coerebinae) from Brazil. Zootaxa 5168:83–91

Parker BB, Duszynski DW (1986) Polymorphism of eimerian oocysts: a dilemma posed by working with some naturally infected hosts. J Parasitol 72:602–604. https://doi.org/10.2307/3281518
Pigliucci M (2001) Phenotypic plasticity: beyond nature and nurture. The Johns Hopkins University Press, Baltimore
Pinto C (1928) Synonymie de quelques especes du genre *Eimeria* (Eimeridia, Sporozoa). C R Seances Soc Biol 98:564–1565
Ray DK (1952) On a new coccidium, *Eimeria sphenocercae* n. sp., from *Sphenocercus sphenurus* (Kokla Green pigeon). J Parasitol 38:546–547
Ridgely RS, Gwynne JA, Tudor G, Argel M (2015) Aves do Brasil: Mata Atlântica do Sudeste. Horizonte, São Paulo
Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542
Sampaio IBM (2002) Estatística aplicada à experimentação animal. FEP MVZ Editora, Belo Horizonte
Sick H (1997) Ornitologia Brasileira. Nova Fronteira, Rio de Janeiro
Silva-Carvalho LM, Genovez-Oliveira JL, Oliveira MS, Oliveira ÁA, Lima VM, Ferreira I, Berto BP (2020) Polymorphism and genetic diversity of *Isospora parnaitatiaiensis* Silva, Rodrigues, Lopes, Berto, Luz, Ferreira & Lopes, 2015 (Eimeriidae) from antbirds (Thamnophilidae) in Brazil. Syst Parasitol 97:847–855. https://doi.org/10.1007/s11230-020-09940-6
Taroda A, Barros LD, Seixas M, Cardim ST, Sasse JP, Minutti AP, Vidotto O, Garcia JL (2020) First molecular detection of Eimeria spp. in eared doves (*Zenaida auriculata*) from Brazil. Semina: Ciências Agrárias 41:1259–1266
Taylor MA, Coop RL, Wall RL (2017) Parasitologia Veterinária. Guanabara Koogan, Rio de Janeiro
Varghese T (1978) *Eimeria waiganiensis* sp. n. from the greenwinged ground dove (*Chalcophaps indica* Linnaeus) and the magnificent ground pigeon (*Otidiphaps nobilis* Gould) in Papua New Guinea. J Parasitol 64:312–314. https://doi.org/10.2307/3279680
Varghese T (1980) Coccidian parasites of birds of the avian order Columbiformes with a description of two new species of *Eimeria*. Parasitol 80:183–187. https://doi.org/10.1017/S0031182000000640
Wiens JJ (1999) Polymorphism in systematics and comparative biology. Annu Rev Ecol Syst 30:327–362. https://doi.org/10.1146/annurev.ecolsys.30.1.327
Williams RB, Thebo P, Marshall RN, Marshall JA (2010) Coccidian oöcysts as type–specimens: long–termstorage in aqueous potassium dichromate solution preserves DNA. Syst Parasitol 76:69–76. https://doi.org/10.1007/s11230-010-9234-2
Yabsley MJ, Bailey K, Adams HC (2015) A New Species of *Eimeria* (Apicomplexa: Eimeriidae) from the mourning dove, *Zenaida macroura* (Columbiformes: Columbidae). Comp Parasitol 82:231–234. https://doi.org/10.1654/4769.1
Yang R, Brice B, Elliot A, Ryan U (2016) Morphological and molecular characterization of *Eimeria labbeana*-like (Apicomplexa:Eimeriidae) in a domestic pigeon (*Columba livia domestica*, Gmelin, 1789) in Australia. Exp Parasitol 166:124–130. https://doi.org/10.1016/j.exppara.2016.04.009
Yang R, Brice B, Berto BP, Ryan UM (2020) Morphological and genetic characterization of *Eimeria chalcoptereae* n. sp. (Apicomplexa: Eimeriidae) in a common bronzewing pigeon (*Phaps chalcoptera*) (Latham, 1790) in Western Australia. Parasitol Res 119:3729–3737. https://doi.org/10.1007/s00436-020-06844-8