Observations on Some Avian Coccidia (Apicomplexa: Eimeriidae) in Amazonian Brazil

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Oocysts of Eimeria porphyrulae n.sp. are described in faeces of Porphyrula martinica (Aves: Gruiformes: Rallidae). They are ellipsoidal to oval, 22.4 x 17.7 (20.0-23.7 x 16.2-18.7) µm, shape-index (length/width) 1.3. Oocyst wall about 1.25 µm thick, colourless, with two layers: inner one prominently striated. Micropyle and sub-micropylar granule present: no oocyst residuum. Sporocysts 17.5 x 9.0 (17.0-19.0 x 8.0-10.0) μ m, shape-index 1.9, with inconspicuous Stieda/sub-Stieda bodies. Sporocyst residuum of scattered granules, sometimes a compact mass: sporozoites with two refractile bodies. Eimeria crypturelli n.sp. is described in faeces of Crypturellus soui (Tinamiformes: Tinamidae). Oocysts ellipsoidal-oval, 20.75 x 14.5 (17.5-25.0 x 11.25-21.25) µm, shape-index 1.4. Oocyst wall about 1.25 µm thick and bi-layered: inner layer faintly striated. Micropyle present, with oocyst residuum immediately below: single polar body rarely present. Sporocysts 13.0 x 7.5 (12.5-13.75 x 7.5-8.1) µm, shape-index 1.7, with a Stieda body but seemingly no sub-Stieda. Sporocyst residuum compact: sporozoites with two refractile bodies. Isospora cacici n.sp. is recorded from faeces of Cacicus cela cela (Passeriformes: Icteridae). Oocysts subspherical-spherical, 26.5 x 23.7 (22.5-27.5 x 20.0-26.2) µm, shape-index 1.1. Wall a single, colourless layer about 1.5 µm thick. No micropyle or oocyst residuum: 1-2 polar bodies. Sporocysts ellipsoidal, 17.7 x 12.5 (17.5-18.75 x 11.25-13.75) μm , shape-index 1.4, with pronounced Stieda/sub-Stieda bodies: residuum compact and sporozoites with two refractile bodies. Isospora thraupis n.sp. is described from faeces of Thraupis palmarum melanoptera (Passeriformes: Thraupidae). Oocysts subspherial-spherical, 19.9 x 19.0 (18.7-21.2 x 18.75-20.0) µm, shape-index 1.0. Wall about 0.6 µm thick, smooth, colourless and a single layer: no micropyle, oocyst residuum or polar bodies. Sporocysts 14.2 x 9.2 (13.7-16.2 x 8.7-10.0) µm, shape-index 1.5: Stieda/sub-Stieda bodies inconspicuous. Residuum compact: sporozoites with two refractile bodies.

Key words: Eimeria porphyrulae n.sp. - Eimeria crypturelli n.sp. - Eimeria vitellini - Isospora cacici n.sp. - Isospora thraupis n.sp. - Isospora albicollis - birds - Brazil

The faeces of two recently captured adult specimens of *Porphyrula martinica* (Gruiformes: Rallidae) were found to contain abundant coccidial oocysts, considered to be those of a hither-to unrecorded species of *Eimeria* and described below.

During an examination of captive birds in the Parque Zoobotânico of the Companhia Vale do Rio Doce, Serra dos Carajás, State of Pará, other oocysts were found in single, adult examples of Crypturellus soui (Tinamiformes: Tinamidae), Thraupis palmarum melanoptera (Passeriformes: Thraupidae) Dendrocincla merula and Xiphorhynchus guttatus (Passeriformes: Dendrocolaptidae); two adult Cacicus cela cela (Passeriformes: Icteridae) and five of six adult Xiphor-

hynchus spixii. Among these parasites there are considered to be a further new species of *Eimeria*, and two new species of *Isospora*. These are described below.

A new host record is given for Eimeria vitellini Lainson, Costa & Shaw, 1990, and new information is added to this parasite's previous description. Problems in the taxonomy of avian coccidia are discussed, with particular reference to the genus Isospora.

MATERIALS AND METHODS

Faecal suspensions were made in a 2.0% (w/v) aqueous solution of potassium dichromate (K₂Cr₂O₇) and maintained in loosely covered Petri-dishes at approximately 24-26°C. With the exception of material from Crypturellus, sporulation time of the parasites was ascertained following bi-daily examination of the oocysts.

Fifty oocysts of each parasite were measured, using an ocular micrometer, x 8 eyepieces and a x 100 neofluar objective. Line-drawings were

Grant 035693 Wellcome Trust, London Received 9 December 1993 Accepted 21 March 1994 based on a combination of direct observations made on the oocysts being measured and photomicrographs. The latter were prepared using a Zeiss Photomicroscope III and Kodak TMX 402 film.

All measurements are in micrometers (µm) and are given as means, with the range in parentheses, followed by the shape-index (= ratio of length/width).

RESULTS

Eimeria porphyrulae n.sp. (Figs 1-7; 24)

Diagnosis: oocysts ellipsoidal to oval (eggshaped), 22.4 x 17.7 (20.0-23.7 x 16.2-18.7), shape-index 1.3 (1.1-1.4). The oocyst wall is approximately 1.25 thick, colourless and of two layers: an outer smooth one and an inner, slightly thicker one which is prominently striated. There is a distinct micropyle at the more narrow pole, where the wall gradually becomes thinner. At the thinnest point it appears to be of a single layer and is slightly raised into a small cap of about 2.5-3.0 in width, immediately under which there is a single spherical-oval globule measuring 2.0-3.0. There is no oocyst residuum and, unless the micropylar granule is considered as such, no polar body. The sporocysts are an elongate pearshape, measuring $17.5 \times 9.0 (17.0-19.0 \times 8.0-19.0 \times 10^{-19.0})$ 10.0), shape-index 1.9 (1.8-2.1): there is a delicate Stieda body, and a sub-Stieda body of about 2.0 in width and 1.5 in depth. The sporocyst residuum is most frequently seen as refractile granules which are scattered widely around the sporozoites, but may sometimes be clumped at the broader end of the sporocyst or between the two sporozoites. These possess two refractile bodies, anterior and posterior to the nucleus.

Type host: Porphyrula martinica (Linn.) (Aves: Gruiformes: Rallidae): local name "frango d'água".

Location in host: not ascertained. Probably the small intestine. Oocysts described in the faeces.

Sporulation: exogenous: oocysts mature in two days at 24-26°C.

Type material: oocysts in 10.0% formol-saline and held in the Department of Parasitology, Instituto Evandro Chagas, Belém, PA, Brazil. Repository No. 503.

Type locality: State of Pará, north Brazil.

Prevalence: uncertain: both of the specimens of P. martinica examined were infected.

Pathogenicity: both birds appeared healthy, and with faeces of normal consistency, during ten weeks of observations. Faeces were examined biweekly and oocysts were present on all occasions.

Etymology: the specific name is derived from the generic name of the avian host, Porphyrula.

Eimeria crypturelli n.sp. (Figs 8-10; 23)

Diagnosis: oocysts ellipsoidal to oval, 20.75 x 14.5 (17.5-25.0 x 11.25-21.25), shape-index 1.4 (1.1-1.6). The oocyst wall is about 1.25 thick and composed of two layers: an outer thinner and smooth one, which is colourless or faintly greenish, and an inner thicker one which is yellowish and faintly striated. There is a flat, caplike micropyle at the narrower end of the oocyst, where the wall is much thinner: immediately below there is an oocyst residuum composed of a variable number of spherical globules. On rare occasions a single polar body may be present, variably situated within the oocyst. Sporocysts are an elongate pear-shape, 13.0 x 7.5 (12.5-13.75 x 7.5-8.1), shape-index 1.7 (1.6-1.8): at the pointed end there is a very delicate Stieda body, but a sub-Stieda body is seemingly absent. The sporocyst residuum is usually a bulky ellipsoidal mass lying between the two sporozoites, which possess both anterior and posterior refractile bodies.

Type host: Crypturellus soui (Hermann) (Aves: Tinamiformes: Tinamidae): local name "inambu".

Location in host: uncertain: probably the small intestine. The oocysts were described from the faeces.

Sporulation: exogenous: maturation time not ascertained.

Type material: oocysts in 10.0% formol-saline and held in the Department of Parasitology, Instituto Evandro Chagas, Belém, PA, Brazil. Repository No. 70.

Type locality: Serra dos Carajás, State of Pará, north Brazil (6.0°S-50.18'W).

Prevalence: unknown.

Pathogenicity: unknown. The infected bird appeared to be in perfect health.

Etymology: the specific name is derived from the generic name of the avian host, Crypturellus, in which the parasite was found.

Isospora cacici n.sp. (Figs 11-17; 25)

Diagnosis: oocysts subspherical to spherical, 26.5 x 23.7 (22.5-27.5 x 20.0-26.25), shape-index 1.1 (1.0-1.2). Oocyst wall a single, colourless, smooth layer approximately 1.5 thick: no micropyle or oocyst residuum, but 1-2 smooth, spherical-ellipsoidal polar bodies of variable size (2.75; 2.5 x 1.25). Sporocysts ellipsoidal, 17.7 x 12.5 (17.5-18.75 x 11.25-13.75), shape-index 1.4 (1.2-1.5), with a prominent stopper-like Stieda/sub-Stieda body. Sub-Stieda body measuring about 2.0 in width and 1.0 in depth. Sporocyst residuum bulky, compact, spherical-ellipsoidal and composed of a large number of spherules and

granules. Sporozoites with both anterior and posterior refractile bodies.

Type host: Cacicus cela cela (Linn.) (Aves: Passeriformes: Icteridae): local name "xexéu". Possibly also in Dendrocincla merula, Xiphorhynchus guttatus and X. spixii (Passeriformes: Dendrocolaptidae).

Location in host: not ascertained: probably the small intestine. Oocysts described in the faeces.

Sporulation: exogenous: oocysts complete maturation after five days at 24-26°C.

Type material: oocysts in 10.0% formol-saline and held in the Department of Parasitology, Instituto Evandro Chagas, Belém, PA, Brazil. Repository No. 174.

Type locality: Serra dos Carajás, Pará, north Brazil.

Prevalence: unknown.

Pathogenicity: none of the infected birds showed signs of illness, and their faeces were of normal appearance.

Etymology: the specific name is derived from the generic name of the avian host, Cacicus, in which the parasite was first encountered.

Both specimens of Cacicus c. cela infected with I. cacici n.sp., were also passing oocysts of a different type (Fig. 22). Morphologically these were indistinguishable from Isospora albicollis Lainson & Shaw, 1989, described from Turdus albicollis Passeriformes: Turdidae), in the State of Pará, Brazil. Further comments on this finding are given below.

Isospora thraupis n.sp. (Figs 18-21; 26)

Diagnosis: oocysts subspherical to spherical, 19.9 x 19.0 (18.7-21.2 x 18.75-20.0), shape-index 1.0 (1.0-1.1). Oocyst wall about 0.6 thick, smooth, colourless and of a single layer. No micropyle, oocyst residuum or polar bodies. Sporocysts pear-shaped, 14.2 x 9.2 (13.7-16.2 x 8.7-10.0), shape-index 1.5 (1.3-1.7). Pointed end of sporocyst with a delicate Stieda body and a small sub-Stieda body measuring about 1.5 in width and 0.75 in depth. Sporocyst residuum a compact mass of spherules and granules, usually lying on the four sporozoites, which resemble a "hand" of bananas. Sporozoites with both anterior and posterior refractile bodies.

Type host: Thraupis palmarum melanoptera (Sclater) (Aves: Passeriformes: Thraupidae). Local name "sanhaçu".

Location in host: uncertain: oocysts described from the faeces.

Sporulation: exogenous: oocysts mature in three days at 24-26°C.

Type material: oocysts preserved in 10.0% formol-saline and held in the Department of

Parasitology, Instituto Evandro Chagas, Belém, PA, Brazil. Repository No. 304.

Type locality: Serra dos Carajás, Pará, north Brazil.

Prevalence: unknown.

Pathogenicity: unknown: the single specimen of T. p. melanoptera examined appeared quite healthy and the faeces were of normal consistency.

Etymology: the specific name of the parasite is derived from the generic name of the avian host, Thraupis.

Eimeria vitellini Lainson, Costa & Shaw, 1989

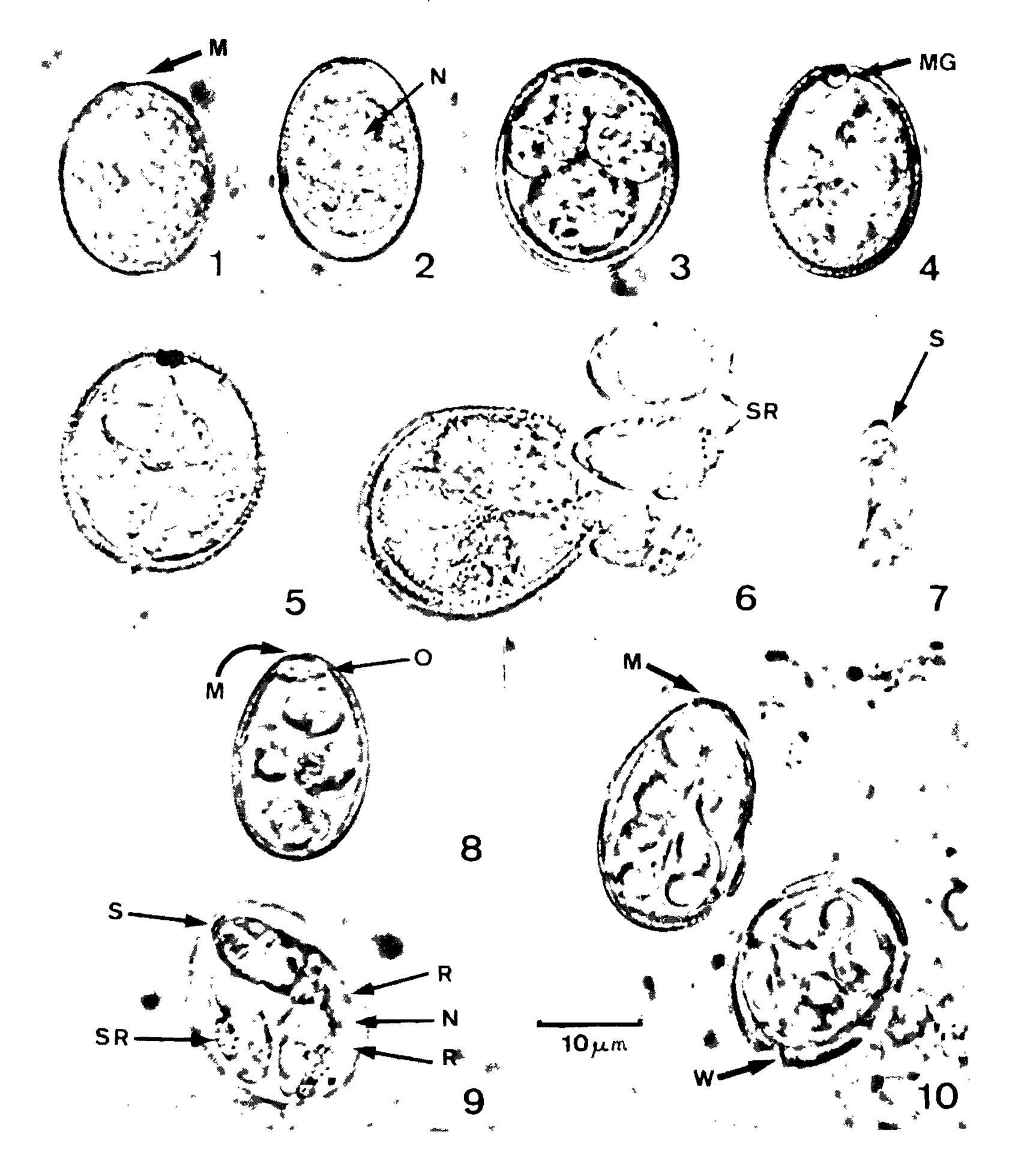
This coccidian was originally described from the faeces of two out of six specimens of the sulphur and white-breasted toucan, Ramphastos v. vitellinus Lichtenstein (Piciformes: Ramphastidae), housed in the Museu Paraense Emílio Goeldi, Belém, PA, Brazil. A recent examination of other birds from the same zoological gardens has now revealed the presence of the parasite in faeces from seven out of nine white-breasted toucans, R. tucanus tucanus (Linn.) No additional information can be added to the original description of *E. vitellini*, but it was possible to verify the sporulation time of approximately 48 hr at 24-26°C and the apparent lack of pathogenicity on the part of the parasite. Although the infection-rate in the captive birds was remarkably high, this cannot be taken as an indication of the prevalence of infection in Nature.

DISCUSSION

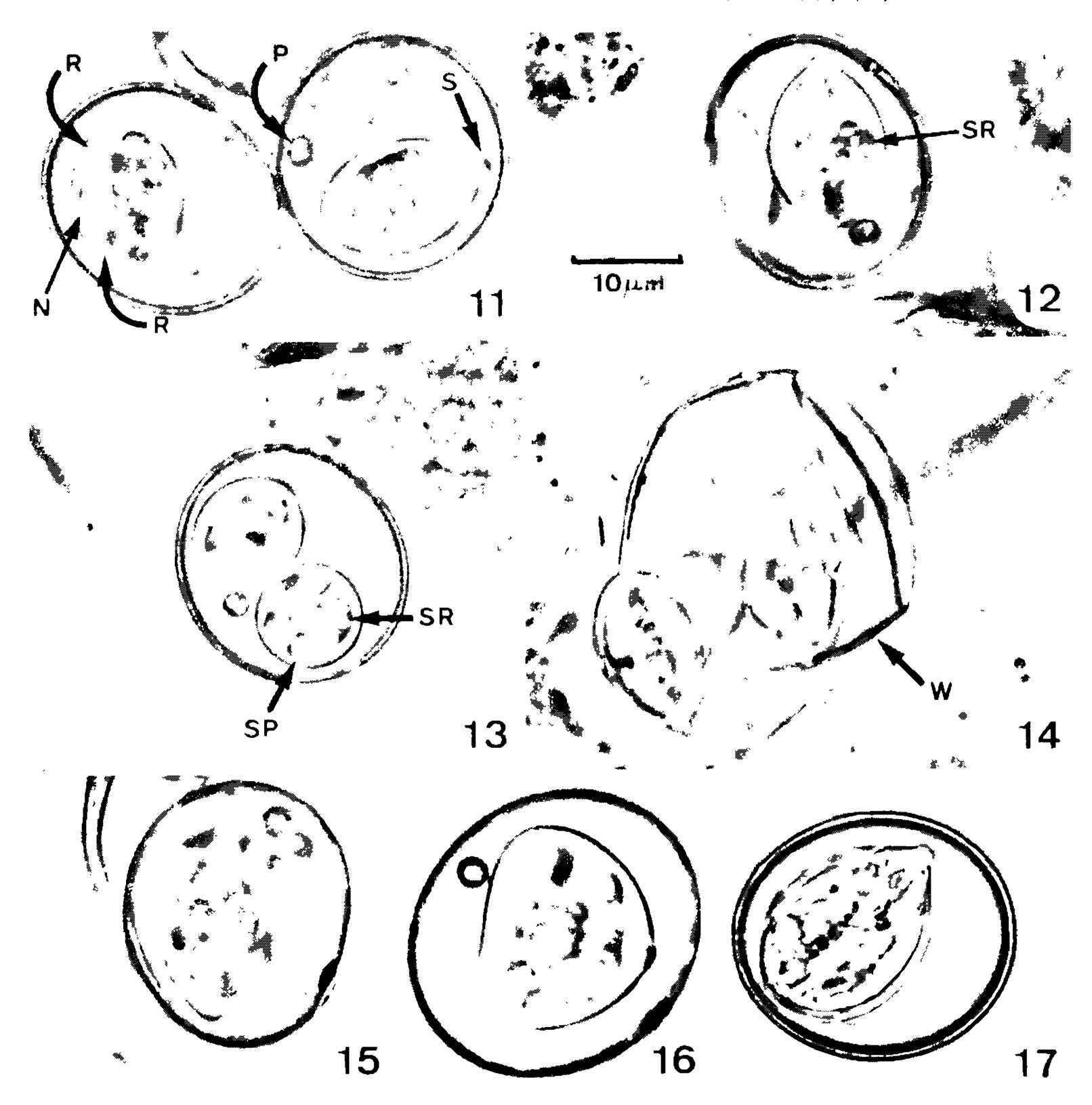
Simultaneous infection with more than one coccidial parasite is of frequent occurrence in birds (Grulet et al. 1982). As the timing of oocyst production for the different species is unlikely to be coincidental, such mixed infections may not be detected on the examination of a single faecal sample. It is possible, therefore, that some of the above-mentioned birds harboured other coccidia in addition to those described, for conditions did not allow the collection of more than one faecal specimen.

Exceptions were the two Porphyrula martinica, faeces of which were repeatedly examined, at various times of day, during a period of ten weeks. The only oocysts found were those of Eimeria porphyrulae.

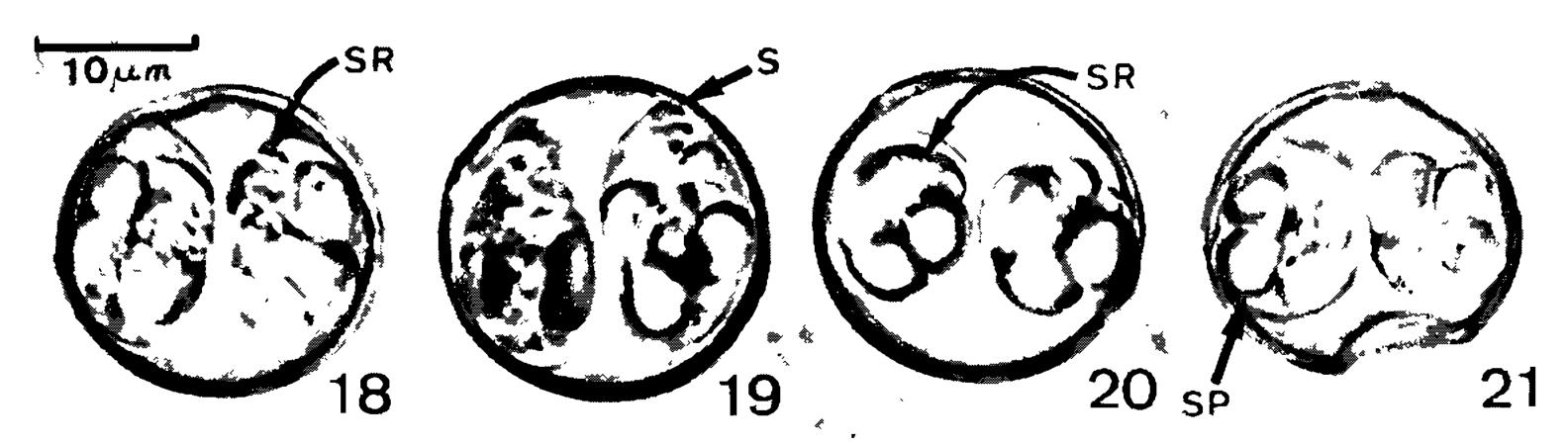
The vexed question of the taxonomy of morphologically indistinguishable or very similar oocysts in different avian genera, particularly within the Passeriformes, might in some cases be resolved by future, carefully controlled cross-infection experiments. This seems a monumental task, however, especially when dealing with the parasites of wild birds, hand-reared specimens of which are not exactly a readily obtainable commodity. Modern biochemical, serological and molecular-biology methods (isoenzymes, monoclonal antibodies and DNA probes) are likely to become of increasing importance, and are possib-



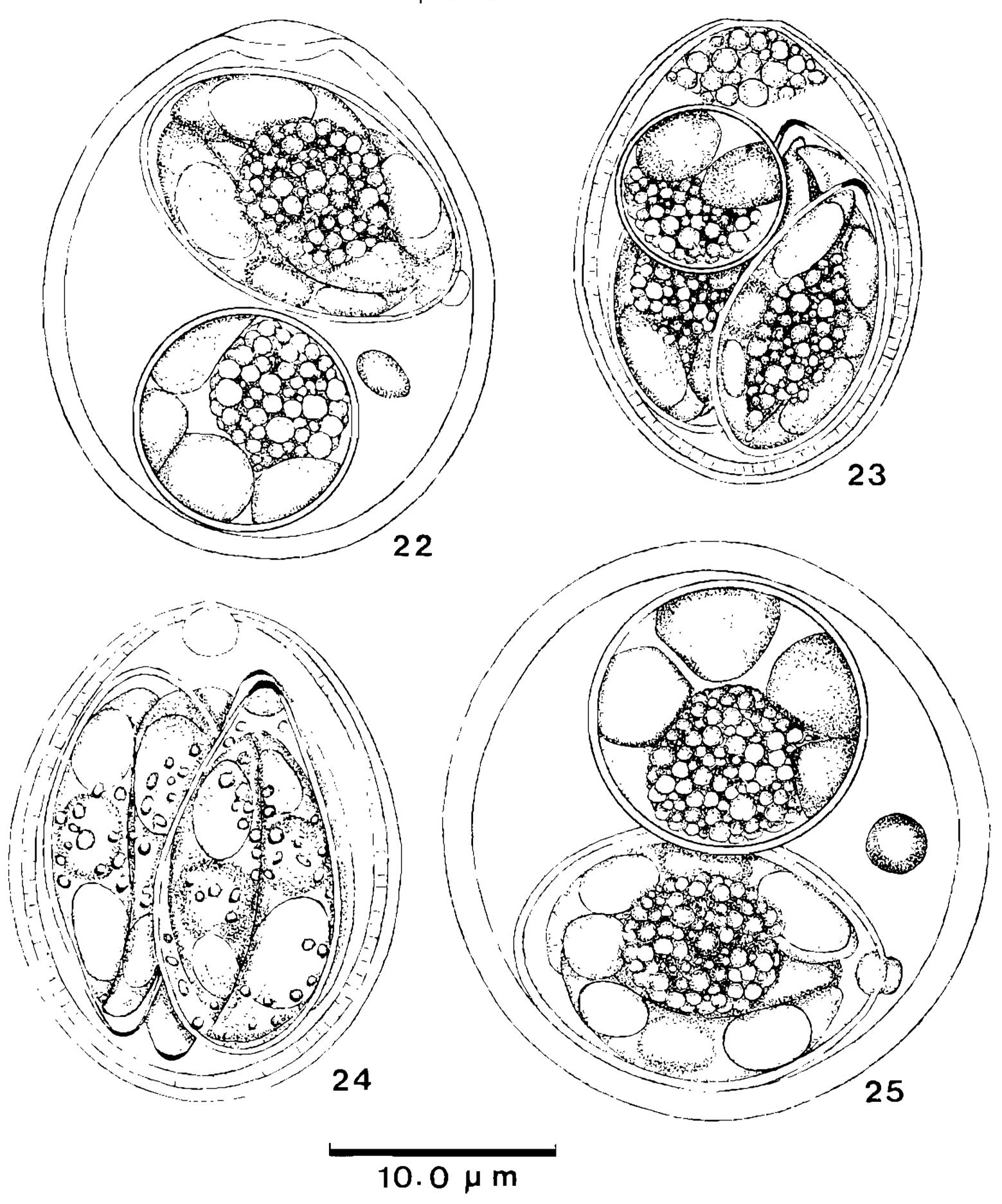
Figs 1-7: Eimeria porphyrulae n.sp. Development of oocysts in faeces of the rail, Porphyrula martinica. Fig. 1: in newly passed faeces: sporont filling the oocyst, and micropyle already visible. Fig. 2: at 24 hr: sporont condensed to a central mass. Fig. 3: at 36 hr: formation of sporoblasts and the sub-micropylar granule. Fig. 4: at 48 hr: mature oocyst with fully formed sub-micropylar granule. Figs 5-6: highly flattened oocysts, showing the pitted bi-layered wall, very pronounced sub-micropylar granule and freed sporocysts. Fig. 7: freed sporocyst with well defined Stieda/sub-Stieda bodies. Figs 8-10: Eimeria crypturelli n.sp. Mature oocysts in faeces of the tinamou, Crypturellus soui. Fig. 8: normal appearance, with conspicuous sub-micropylar oocyst residuum. Figs 9-10: crushed and broken oocysts, showing more clearly the sporocysts and the pitted, bi-layered oocyst wall. M = micropyle; MG = micropylar granule; N = nucleus; O = oocyst residuum; R = refractile body; S = Stieda/sub-Stieda bodies; SR = sporocyst residuum; W = separated layers of oocyst wall.



Figs 11-15: Isospora cacici n.sp. in faeces of the "xexéu", Cacicus cela cela. Figs 11-13: normal, mature oocysts. Fig. 14: crushed oocyst, showing the single-layered wall. Fig 15: aberrant oocyst with one normal sporocyst and two small ones. Figs 16-17: abnormal oocysts of Isospora, possibly I. cacici in Xiphorhynchus spixii. The faeces of both birds contained a variety of deformed oocysts, including those simulating Caryospora (Fig 17). N = nucleus of sporozoite; P = polar body; S = Stieda/sub-Stieda bodies; SP = sporozoite; SR = sporocyst residuum; R = refractile body; W = broken, single-layered oocyst wall.



Figs 18-21: Isospora thraupis n.sp. Mature occysts in the faeces of the "sanhaçu", Thraupis palmarum melanoptera. S = stieda body; SP = sporozoite; SR = sporocyst residuum.



Figs 22-25: line-drawings of mature oocysts from some birds of Amazonian Brazil. Fig. 22. Isospora sp., from Cacicus cela cela: morphologically indistinguishable from Isospora albicollis Lainson & Shaw, described from Turdus albicollis. Fig. 23: Eimeria crypturelli n.sp., from Crypturellus soui. Fig. 24: Eimeria porphyrulae n.sp., from Porphyrula martinica. Fig. 25: Isospora cacici n.sp., from Cacicus cela cela.

ly the ultimate answer to the problem (Nakamura et al. 1991, Lindsay et al. 1991, Stucki et al. 1993, Gajadhar 1993).

In these studies I have favoured the generally accepted view that morphologically indistinguishable oocysts in closely related birds (e.g. in species or subspecies, within a genus) are likely to belong to the same parasite, and that oocysts

from birds which are taxonomically widely separated (e.g. from different families or higher taxa) are most likely to represent different coccidial species.

The consensus of opinion supports the view that members of the genus *Eimeria* are, in fact, highly specific. Cross-transmission is virtually non-existent between families and remains rare

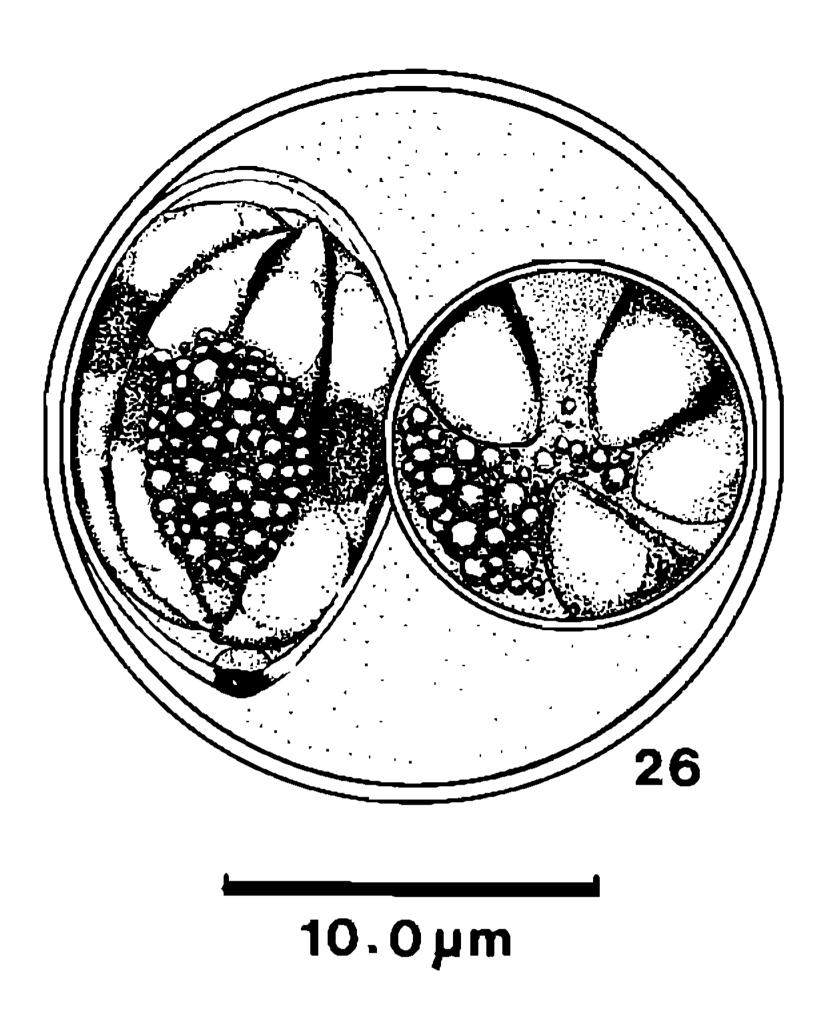


Fig. 26: Isospora thraupis n.sp., from Thraupis palmarum melanoptera.

between different genera. Some species of this parasite, however, have been shown to complete their life-cycle in closely related animals (species or subspecies within a given genus), and the finding of *E. vitellini* in two species of the toucan, *Ramphastos* (present and previous papers), comes as no great surprise.

The situation regarding Isospora is more complicated and, due to the wide range of "alternative hosts" recorded for some avian species of this coccidian, notably I. lacazei and I. volki, there developed the probably erroneous impression that the genus has a much lower degree of specificity than does Eimeria. Unfortunately, many early workers chose to disregard what they considered to be minor differences in the oocyst morphology of "L. lacazei" from different birds, with the result that some 40 or more species of passerines became listed as hosts of this parasite.

Anwar (1966) discussed this problem and, in a study of isosporan oocysts in sparrows (Passer d. domesticus), greenfinches (Chloris chloris) and chaffinches (Fringilla coelebs), concluded that the oval "form" of I. lacazei described in these birds by previous workers was in fact another species, which he named I. chloridis. He considered that the oocysts of I. lacazei are the spherical ones seen in mixed infections of the two parasites. Furthermore, he was able to correlate the two different oocysts with differences in

the endogenous development of these coccidia in the host intestine. Finally, Levine (1988) clearly expresses strong doubts regarding the lack of host-specificity on the part of *I. lacazei* when he refers only to the goldfinch, *Carduelis carduelis*, as its host - this being the type host of the parasite in the original description by Labbé (1893). This extreme view presumably casts doubt on the validity of Anwar's identification of the morphologically similar, spherical oocysts of sparrows, greenfinches and chaffinches as *I. lacazei*.

As far as I am aware, no coccidia has previously been described from *Porphyrula martinica*. A number have been recorded from other birds of the family Rallidae, however, and need to be compared with *E. porphyrulae* n.sp.

Léger and Hesse (1922) gave the name of Jarrina paludosa to an eimerian they found in the coot Fulica atra and the moorhen Gallinula chloropus in France, and Hoare (1933) amended the name to Eimeria paludosa. McAllister and Upton (1990) found the same parasite in the American coot, Fulica a. americana and redescribed the oocysts. These are 16.5×12.6 (15-23 x 11-14), with a single-layered, pitted (striated) wall of about 1.0. There is a micropyle, 5.3 wide, beneath which there is a single polar granule: this disintegrates, however, on sporulation, to give "several to many smaller granules". The sporocysts are $10.8 \times 6.2 (10-12 \times 5-7)$, and the sporulation time of the oocysts is given as 5-7 days at 23°C. The authors compared E. paludosa with E. fulica Matschoulsky 1941, and E. polycephali Yakimoff & Matschoulsky 1939, from Fulica atra and Porphyrio coerulens, respectively, and considered these to be synonyms. E. porphyrulae n.sp., is readily differentiated from E. paludosa by its much larger oocysts and sporocysts, much smaller micropyle, a sub-micropyle granule which does not disintegrate following sporulation (Figs 4, 5) and its much shorter sporulation time of only two days.

E. mongolica Matschoulsky, 1941 was described from F. atra and is clearly separated from E. porphyrula by oocysts which measure only 14.4 x 12.5 and bear no micropyle.

Finally, the oocysts of *E. alakuli* figured by Rakhmatullina-Batyshrina and Svanbaev (1972) measure 25.2 x 19.6: the sporocysts only 11.2 x 5.6. The wall appears to be of a single layer; there is no sub-micropylar granule and the micropyle is much larger and more conspicuous than that of *E. porphyrulae*. The parasite was described from *Fulica atra*.

Only one Eimeria species has been previously recorded in the order Tinamiformes, family Tinamidae, namely E. rhynchoti Reis & Nobrega, 1936 in Rhynchotus rufescens from the State of São Paulo, Brazil. It differs from E. crypturelli n.sp., in its larger, subspherical oocysts (average

25.0 x 22.0), presence of a single polar body as opposed to a cluster of submicropyle granules, and absence of a micropyle.

I have no knowledge of an isosporan previously described from Cacicus c. cela, and the only record of Isospora from any member of the family Icteridae appears to be I. divitis Pellerdy, 1967 from the Cuban blackbird, Ptiloxena atroviolacea. Although its oocysts are of a similar size to those of I. cacici n.sp., they have a delicate wall which is easily deformed, no polar bodies and no "stopper-like" Stieda/sub-Stieda body comparable with that of the latter parasite.

Similarly, no *Isospora* species appear to have been previously recorded from members of the family Thraupidae (tanagers) which, like icterids, are restricted to the New World. The delicate Stieda/sub-Stieda bodies of *I. thraupis* n.sp., contrast sharply with the bulky, stopper-like structures seen in *I. cacici*, *I. albicollis* and a number of other avian *Isospora* species.

Having voiced the opinion above, that Isospora oocysts from taxonomically widely separated birds are likely to belong to different species of this genus, I confess my reluctance to give a new name to the parasite which appears to be I. albicollis in Cacicus c. cela (Icteridae), originally described in Turdus albicollis (Turdidae), or to those that appear to be I. cacici in three species of birds of the family Dendrocolaptidae (present observations). Hopefully, further study of these will establish their identity.

In the material from Cacicus c. cela, I came across what appeared to be typical Caryospora oocysts. They were scanty, and a search for further examples for measurement revealed the presence of clearly aberrant oocysts which variably contained a single, normal sporocyst plus one or two dwarf sporocysts, with miniature sporozoites; a single large, deformed sporocyst; or a single, perfectly formed sporocyst with eight sporozoites (Figs 15-17). Based on the dimensions of these forms, and the frequent presence of one or two polar bodies, I conclude that they all represent abnormally developing oocysts of I. cacici. Similar aberrant oocysts were seen in the faeces of one specimen of Xiphorhynchus spixii containing isosporan oocysts morphologically very similar to those of *I. cacici* (Figs 16, 17).

This phenomenon of aberrant oocysts, some of which simulate Caryospora, has also been reported for I. spilogales, of Spilogale putorius ambavalis (Mammalia: Carnivora) by Levine and Ivens (1964); I. rivolta in Canis familiaris (Levine & Ivens 1965); I. suis, in pigs, by Vetterling (1965); I. californica in Peromyscus spp. (Mammalia: Rodentia) by Davis (1967); I. felis in Felis catus (Shah, 1970); and I. lacazei in the sparrow, Passer d. domesticus by Černá (1974). The latter author was probably not deal-

ing with *I. lacazei*, but with one of the other similar *Isospora* species since described in this bird (Grulet et al. 1982). Reasons for this abnormal development of *Isospora* species remain obscure. Its frequent occurence, however, clearly indicates the need for caution in diagnosing *Caryospora* infections in animals (largely reptiles and birds) which are also known to harbour *Isospora* species.

In conclusion, I am acutely concious of the possibility that important additional taxonomic criteria might be obtained from a study of the endogenous stages of the parasites described in this paper, and that some workers even question the desirability of naming coccidia solely on the morphology of their oocysts, however characteristic they may appear to be.

It is as well to remember, however, that our knowledge of the existence of the vast majority of nearly 2000 species within the Eimeriidae is due to exactly such descriptions. In discussing classification of malarial parasites, Garnham (1966) wrote...."A large body of opinion.... has advised delay in order to collect more data" and "It seems that this attitude can be maintained too long...." "....it is probably better to possess a list of names which can be sunk when proof of synonomy is forthcoming...."

Similar sentiments can be applied to the coccidia, and refusal to describe and name oocysts until the entire life-cycle is known tends to sink the organism in question into obscurity, and precludes the stimulus for others to study it.

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