SARCOCYSTIS BOOLIATI N.SP. AND A PARASITE OF UNDETERMINED TAXONOMIC POSITION, *OCTOPLASMA GARNHAMI* N. GEN. N. SP., FROM THE MOONRAT, *ECHINOSOREX GYMNURUS*

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INTRODUCTION

While studying the blood and tissue parasites of some small mammals in West Malaysia, a *Sarcocystis* (in muscle) and a protozoon of undetermined status (in the spleen and liver) were discovered in the moonrat *Echinosorex gymnurus*. These findings were reported in preliminary communications (Dissanaike *et al.*, 1974a and b). It was felt at first that the two organisms were perhaps related and that the stages seen in the liver and spleen were probably developmental stages of the *Sarcocystis*. As there was no clear justification for this on the present evidence, the organisms are described separately. The *Sarcocystis* is described as *Sarcocystis booliati* n.sp. after Mr. Lim Boo Liat without whose assistance these parasites would never have been discovered. The other parasite is named *Octoplasma garnhami* gen. et sp. nov. in honour of Professor P.C.C. Garnham, FRS. This organism, which belongs to the subphylum Apicomplexa Levine, 1970 cannot be fitted into any known subdivision of the subphylum at present.

MATERIALS AND METHODS

The moonrat is an insectivore found in the lowland forests of Malaysia and is a difficult animal to trap. The three moonrats from which the parasites are described were trapped respectively in the Selangor-Gombak Forest Reserve, in Gunong Besut, Perak and in Bukit Lanjau, Selangor.

Blood, and tissue impression smears of muscle and most organs were air dried, fixed in methanol and then stained in Giemsa's stain. Sections of muscle and of organs such as the liver, spleen, kidneys, heart and lymph nodes were fixed in neutral formo-saline, cut at 4-6 μm thickness and stained with haematoxylin, Gomori's trichrome stain or by the Giemsa colophonium method. Faecal samples of the last two moonrats were examined for coccidian oocysts.

Measurements and observations on fresh cysts were made on muscle from the third moonrat. Zoites were studied, measured and photographed from Giemsa-stained material or by phase contrast microscopy. Drawings of the zoites and pseudocysts were made using a M20 Wild microscope and drawing attachment.

RESULTS

Octoplasma garnhami gen. et sp. nov.

Tissue stages of this parasite were seen in the moonrat from the Selangor-Gombak Forest Reserve. These were present as intracellular zoites in monocytes of the liver (Fig. 2) and spleen (Figs. 1, a-c; and 3-7). Each zoite was elongate and banana-shaped, with the anterior end more pointed, and had a more or less central nucleus in which a distinct nucleolus was clearly seen even in dry-fixed material. Zoites were 13 to 17 by 2.5 to 4 μm (N = 16), with a mean of 15.0 ±

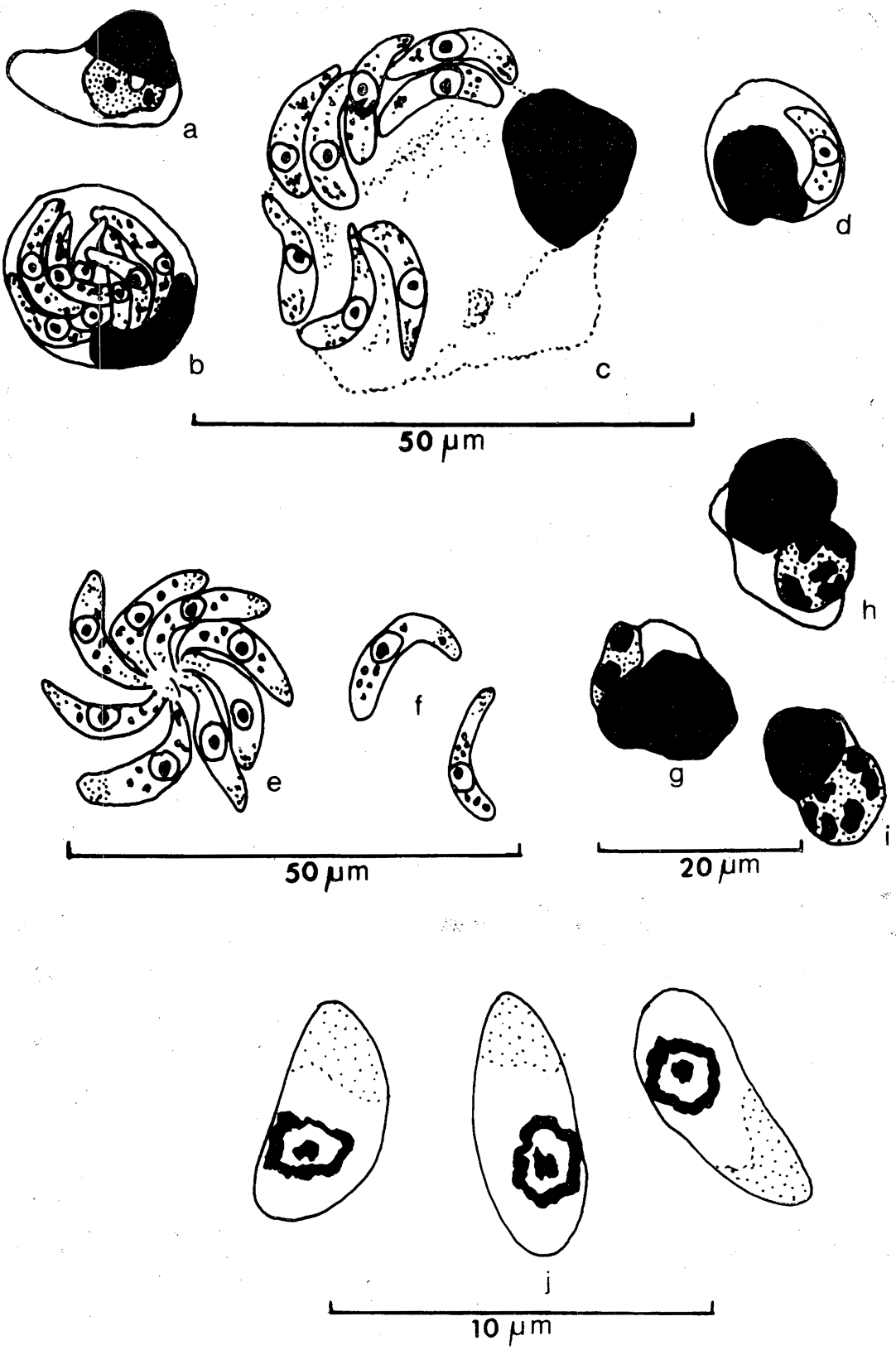
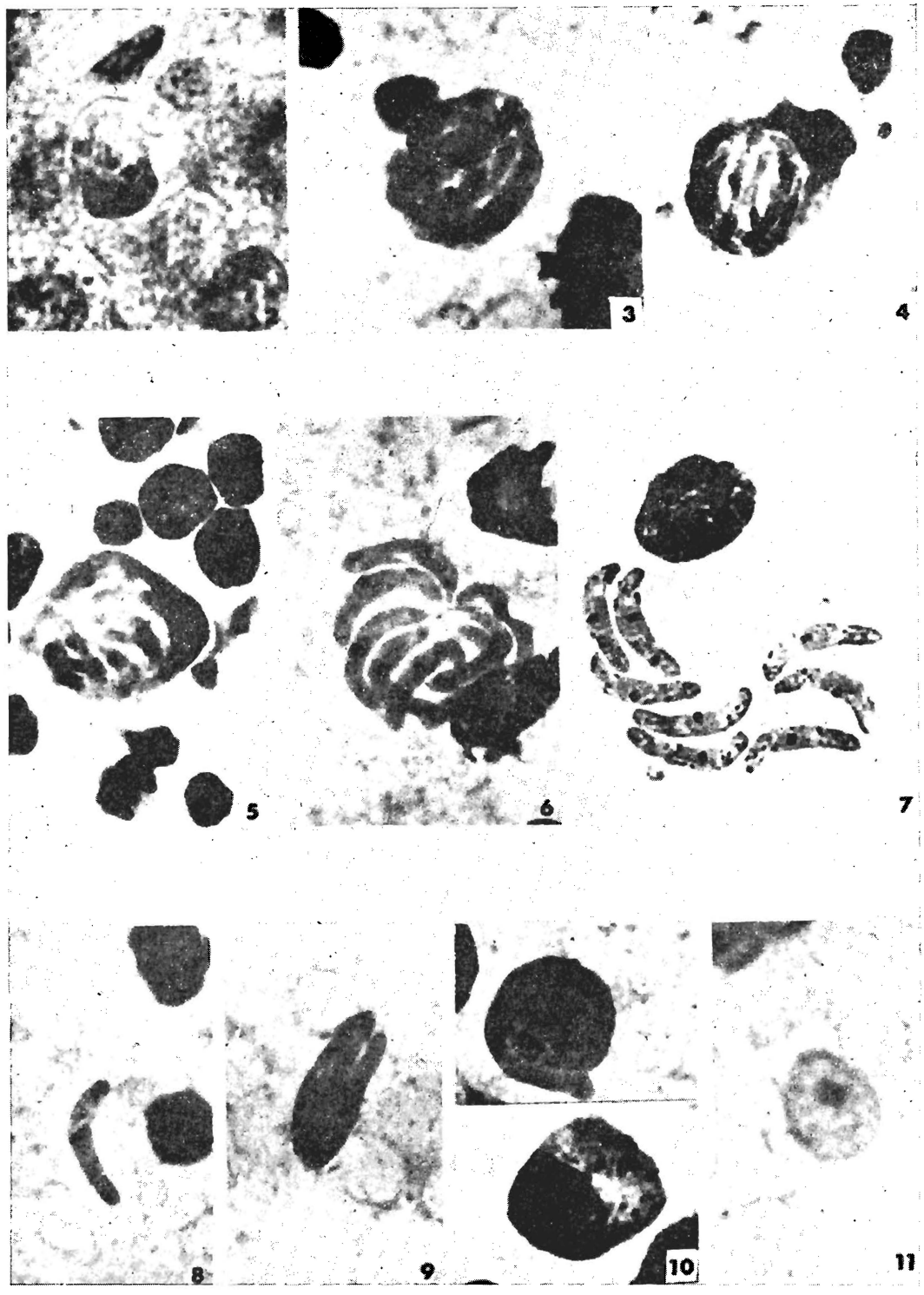


Fig. 1—Camera lucida drawings of *O. garnhami* and *S. booliati* from Giemsa-stained smears. a-f: Stages of *O. garnhami* from spleen of first moonrat. a: Early 2-unclear stage. b, c: Pseudocysts. d: Monocyte with single zoite. e: 8 zoites from ruptured pseudocyst. f: 2 free zoites. g, h, i: 2, 4 and 5-unclear schizonts from spleen of second moonrat. j: Three zoites of *S. booliati* from Miescher's tubule of third moonrat.



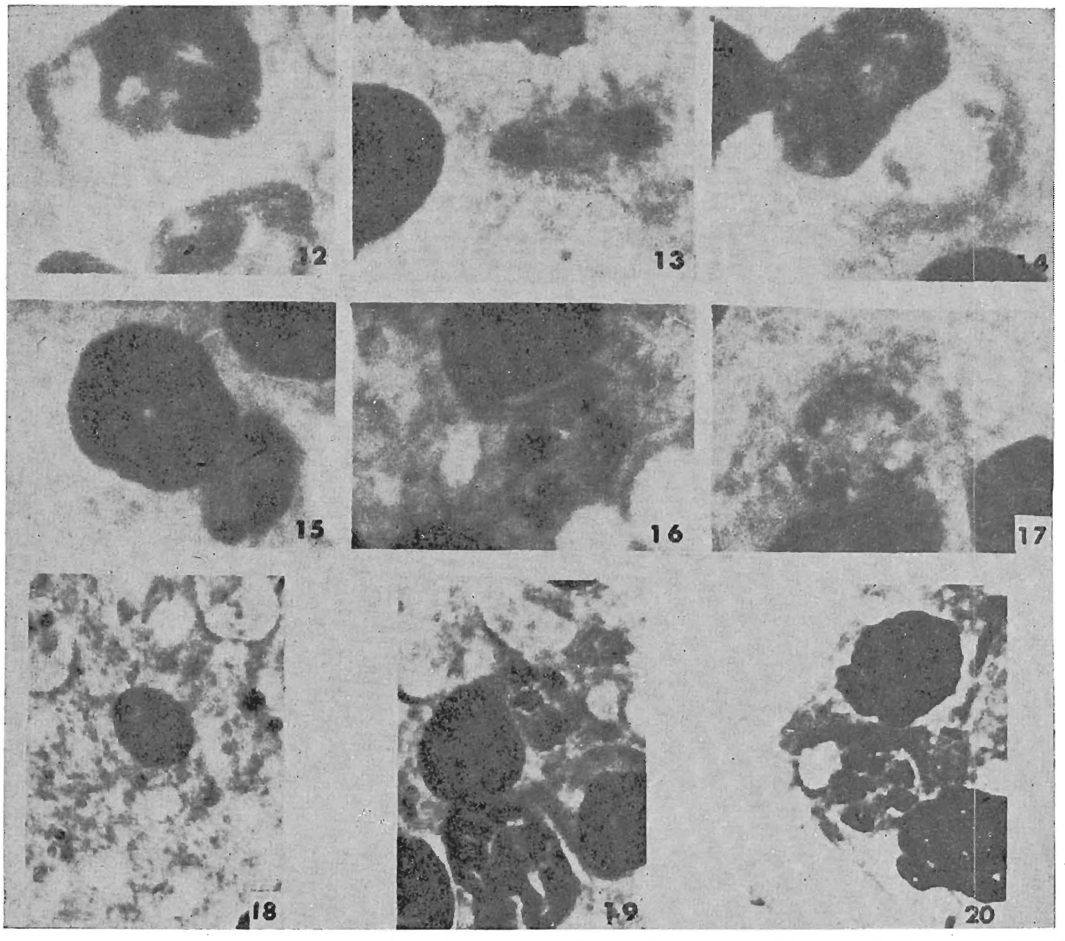
Figs. 2-11—Zoite stages of *O. garnhami* from first moonrat (Giemsa stained). 2 : Pseudocyst from liver section (x 1815). 3 & 6 : Pseudocysts from spleen smear (x 1500). 4, 5, 7 : Pseudocysts from spleen smear (x 1350). 8 : Single free zoite from spleen smear (x 1500). 9 : Dividing zoite from spleen smear (x 1500). 10 : Two intracellular zoite stages from spleen smear (x 1815). 11 : Rounded form, probably early intracellular stage (x 1500).

1.8 by 3.3 ± 1.1 μm in dry-fixed smears and about 10.0 by 2.5 μm in sections. The nucleus of the zoite was 3.0 to 4.0 μm with a mean of 3.7 ± 0.4 μm. The cytoplasm contains several coarse granules.

Some free zoites were also seen in smears, perhaps set free by rupture of pseudocysts during smearing (Fig. 1 e, f and Fig. 8); one of these showed evidence of division by longtitudinal fission (Fig. 9). Several monocytes contained a single zoite (Fig. 1, d and Fig. 10). A few rounded stages with a faint staining nucleus (Fig. 11) were seen. These were probably intracellular early stages.

The muscle of this moonrat was not examined for *Sarcocystis* and no other stages of *O. garnhami* were seen in sections or smears of any of the other organs, nor were any parasites seen in blood films.

In the moonrat from Gunong Besut, smears of the spleen contained numerous schizonts of this parasite with 2 to 7 nuclei (Fig. 1, g, h, i and Figs. 12-20), but none of the



Figs. 12-20—Schizont stages of *O. garnhami* from spleen of second moonrat. Giemsa stained smears. 12 : 2 separate unicellular stages in a monocyte (x 1500). 13 : Binuclear stage (x 1500). 14 : 3-nuclear schizont (x 1500). 15 : 4-nuclear schizont (x 1500). 16 : 5-nuclear schizont (x 1500). 17 : 6-nuclear schizont (x 1500). 18-20 : 3 schizonts (x 1430).

intracellular zoite stages seen in the first moonrat. These were probably early stages in the formation of the 8 zoites seen within host cells of the first moonrat. In this animal, since a possible connection between the intracellular zoites in the liver and spleen and a *Sarcocystis* infection was suspected (Dissanaike *et al.*, 1974a), sections of muscle were examined and showed Miescher's tubules.

The third moonrat, the one from Bukit Lanjau, had no tissue stages in any of the organs examined. However, it had a very heavy *Sarcocystis* infection. No coccidian oocysts were seen in stools of the moonrats.

Sarcocystis booliati n.sp.

Cysts of this parasite are seen in skeletal but not in heart muscle of moonrats from Gunong Besut and Bukit Lanjau. The Miescher's tubules were thin, long (Fig. 21) and cylindroid, up to 4-5 mm in length with tapering extremities (Fig. 22). These tubules were 50 to 110 μm in diameter. The tubules were much longer in the third moonrat, which had the more mature forms. Their maximum diameter was 110 μm, and sections of most muscles examined had more cysts per cross section (Fig. 23) than the other rats.

Sections of the cysts showed a very thin wall (less than 0.5 μm thick) with no evidence of projections from the cyst wall surface (cytophaneres) and no compartmentalization (trabeculae) (Figs. 28 and 29). Longitudinal sections of cysts, however, sometimes showed pseudosegmentation in areas where the muscle fibres had been damaged and hence contracted (Fig. 24). The absence of internal trabeculae was confirmed in sections stained with Gomori's trichrome stain. In sections of the terminal parts of cysts, developing zoites (metrocytes) were seen as light-staining rounded cells (Figs. 25, 26 and 27).

The zoites were small and when examined fresh under phase contrast (Figs. 30 and 31) appeared more elongate and narrower than zoites in dry-fixed smears (Fig. 1, j and Fig. 32). In dry-fixed Giemsa-stained material they were 5 to 8 μm by 2 to 3 μm with a mean of 6.5 ± 0.8 by 2.2 ± 1.2 μm. The nucleus was 2 to 3 μm long with a mean of 2.4 ± 0.1 μm. Each zoite was kidney- or crescent-shaped with the position of the nucleus varying from the posterior end to around the middle of the zoite. Even in Giemsa-stained smears a central darker staining area in the nucleus probably represented the position of the nucleolus. The anterior end of the zoite stained a homogeneous pink while the rest of the cytoplasm had a few granules. Fig. 33 shows some large zoites from cysts of *Sarcocystis* from the muscles of a buffalo for comparison.

DISCUSSION

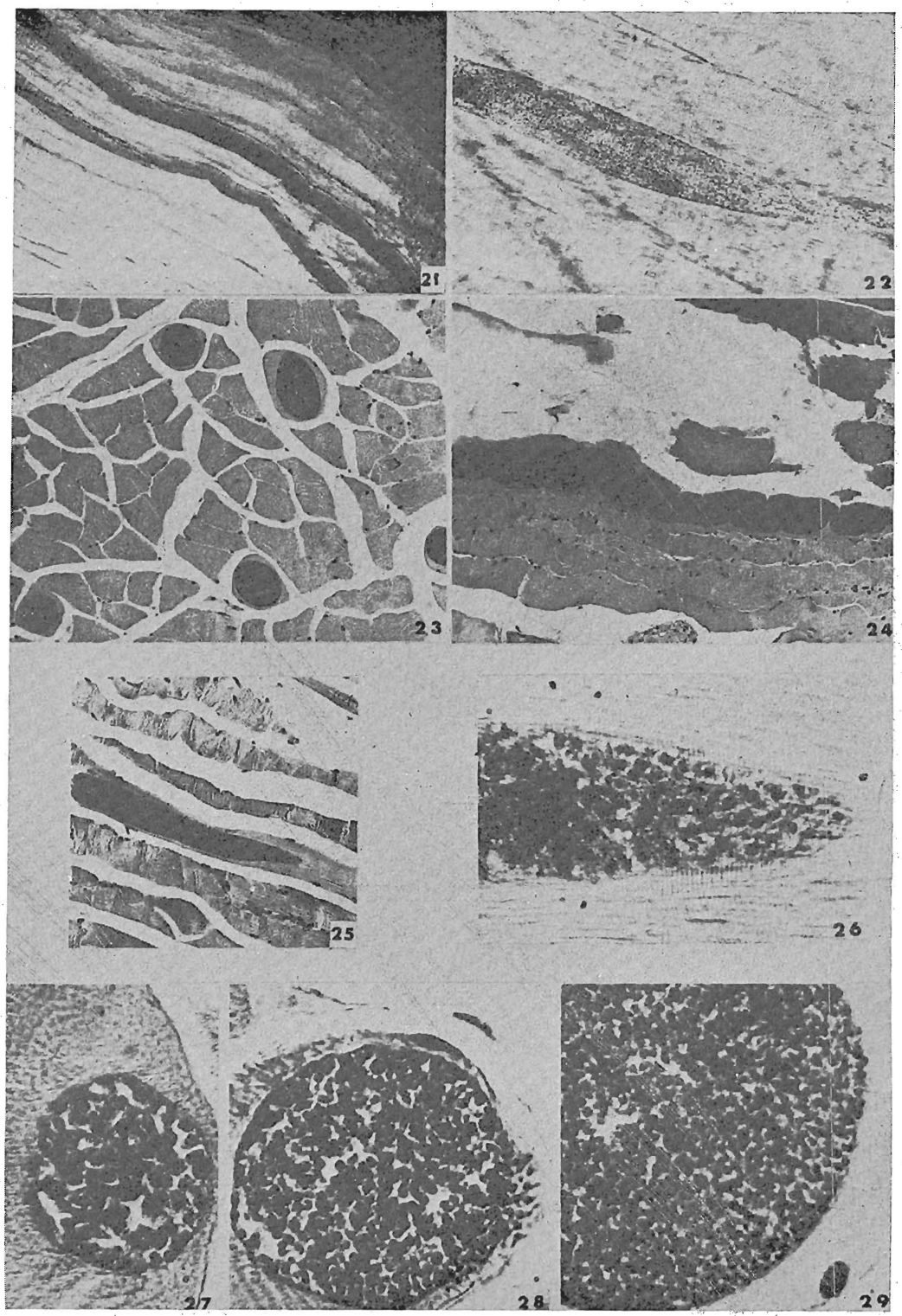
At present the description of any new species of *Sarcocystis* is based on the following:

(i) a new record, especially if the host belongs to a new order or family;

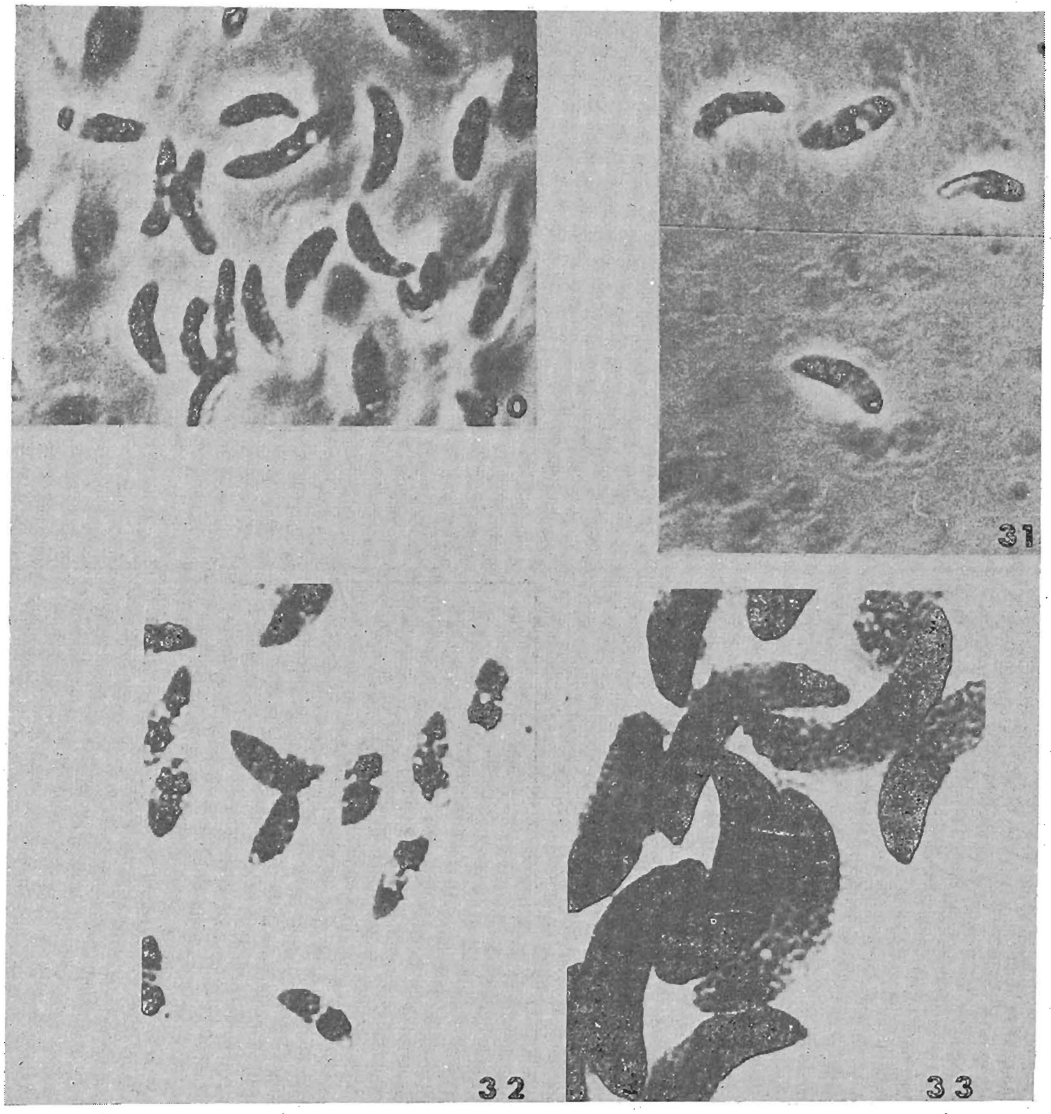
(ii) the structure of the cyst wall, with respect to the presence or absence of trabeculae within and cytophaneres on the cyst wall;

(iii) the shape and size of the zoites, although in less host-specific species this appears to vary with the host in which the parasite develops.

Previous records of *Sarcocystis* from the Insectivora are few. Dollfus (1961) recorded a *Sarcocystis* from *Sorex minutus* in France, and Sebek (1963) reported a *Sarcocystis* which he called an M-organism, in *S. araneus* in Czeckoslovakia. The zoites of the organism were 7-10.5 by 2-3.5 μm. The present species, apart from being in a new host in a different geographic region, has much smaller



Figs. 21-29—Miescher's tubules of *S. booliati* from third moonrat. 21: Unstained cysts from fresh muscle (x 48). 22: Fresh cyst showing tapering end (x 160). 23: T.S. of muscle showing 5 cysts in cross section (x 95). 24: L.S. of cyst showing pseudosegmentation (x 95). 25: L.S. of terminal end of a cyst (x 95). 26: Part of same cyst under oil immersion to show metrocytes (x 950). 27: T.S. of terminal end of a cyst showing metrocytes (x 950). 28 & 29: Transverse section of 2 regions of a cyst under oil immersion, showing very thin cyst wall and small zoites (x 950).



Figs. 30-33—Zoites of *S. booliati* - 30 & 31 : Zoites under phase contrast (x 1420). 32 : Zoites in Giemsa-stained smears (x 1420). 33 : Giemsa stained zoites of *Sarcocystis* from buffalo, for comparison (x 1420).

zoites (5-8 by 2-3 μm). It also differs from other species of *Sarcocystis* recorded from Malaysia by Zaman (1970) from the slow loris, by Zaman and Colley (1972) from the buffalo and by Prathap (1973) from a long-tailed macaque monkey, in having no trabeculae.

It is not possible to assign the parasite from the spleen and liver to any known genus on the basis of the available data. There is no doubt, however, that it belongs to the subphylum Apicomplexa and most probably to the subclass Coccidiasina. The presence of zoites resembling those of *Sarcocystis*

inside lymphoreticular cells of the host suggests a parallel with the pseudocyst stages of *Toxoplasma*. The recent findings of schizonts of *Sarcocystis fusiformis* in many internal organs of calves fed sporocysts of that organism (Fayer and Johnson, 1973 and 1974) and of zoites of *Isospora felis* and *I. rivolta* in many tissues of mice fed with oocysts of these coccidia (Frenkel and Dubey, 1972) suggest that the schizonts seen in the second moonrat and the zoites seen singly and in groups of 8 in the first moonrat may in fact be similar stages of the moonrat *Sarcocystis*. The fact that these zoites in the first moonrat are much larger than the zoites of *S. booliati* is not difficult to explain since Frenkel and Dubey (1972) observed that the zoites in the tissues of mice fed sporocysts of *I. rivolta* were much larger than the sporozoites of *I. rivolta*. Furthermore, it is known that the zoites (cystozoites) of *Toxoplasma gondii* in cysts are smaller than the zoites within pseudocysts (tachyzoites, endozoites). If therefore we consider that the zoites and schizonts in the first two moonrats are in fact developmental stages of *S. booliati*, it would appear that the first moonrat was caught while the parasite was in the early stage with circulating zoites either single or after division into octozoic forms. Also that in the second moonrat the organism had passed this stage and had some already-developed Miescher's tubules together with some schizont stages in the tissues. In the third moonrat the infection may have gone on for so long that no more tissue stages were present. This will explain why heavier infections of the muscle were seen in the third animal, and why on the whole the cysts were much larger and more mature than in the second. Unfortunately, the muscle was not examined for *Sarcocystis* in the first moonrat.

On the other hand, it is probably more likely that the parasite from the tissues of the first and second moonrats is quite distinct for the following reasons:

(a) the zoites in the pseudocysts were much larger than those in the Miescher's tubules of *S. booliati*;

(b) the pseudocysts, when fully mature, invariably contained 8 zoites, a feature not seen so far in the intracellular stage of any known protozoon.

Therefore a new genus is created for this organism, the features of which are at present not clearly defined but include 8 zoites in the intracellular stage (pseudocyst). The schizonts present in the second moonrat might be either earlier stages in the formation of these zoites or perhaps might give rise to merozoites which enter fresh monocytes to become the zoites seen in Fig. 1, d and Fig. 10. These might then divide by binary fission (Fig. 9) to give rise to a group of 8 zoites (Figs. 1, b, c and e and 3-7). The complete life cycle is, however, still obscure. Feeding of muscle, if possible to clean moonrats, and to dogs and cats or other carnivores will eventually help elucidate the life cycle of the *Sarcocystis* and indeed help to decide whether there is in fact a connection between the two organisms.

In Fig. 34, which is self-explanatory, an attempt has been made to summarise the known facts in the life cycles of the two parasites. It also indicates the gaps in our knowledge of them and suggests possible connections between organisms.

The moonrat is an insectivore, and, according to Lim (1967), feeds mainly on earthworms and arthropods. One of these invertebrates may therefore be one of the hosts in the life cycle of *O. garnhami* if it proves to be unrelated to *S. booliati*. The vector may even be an ectoparasitic mite or tick (Fig. 34). The dog has been shown in Fig. 34 as a possible definitive host merely because so far it is one of the proved hosts of a *Sarcocystis* (Fayer and Johnson, 1973 and

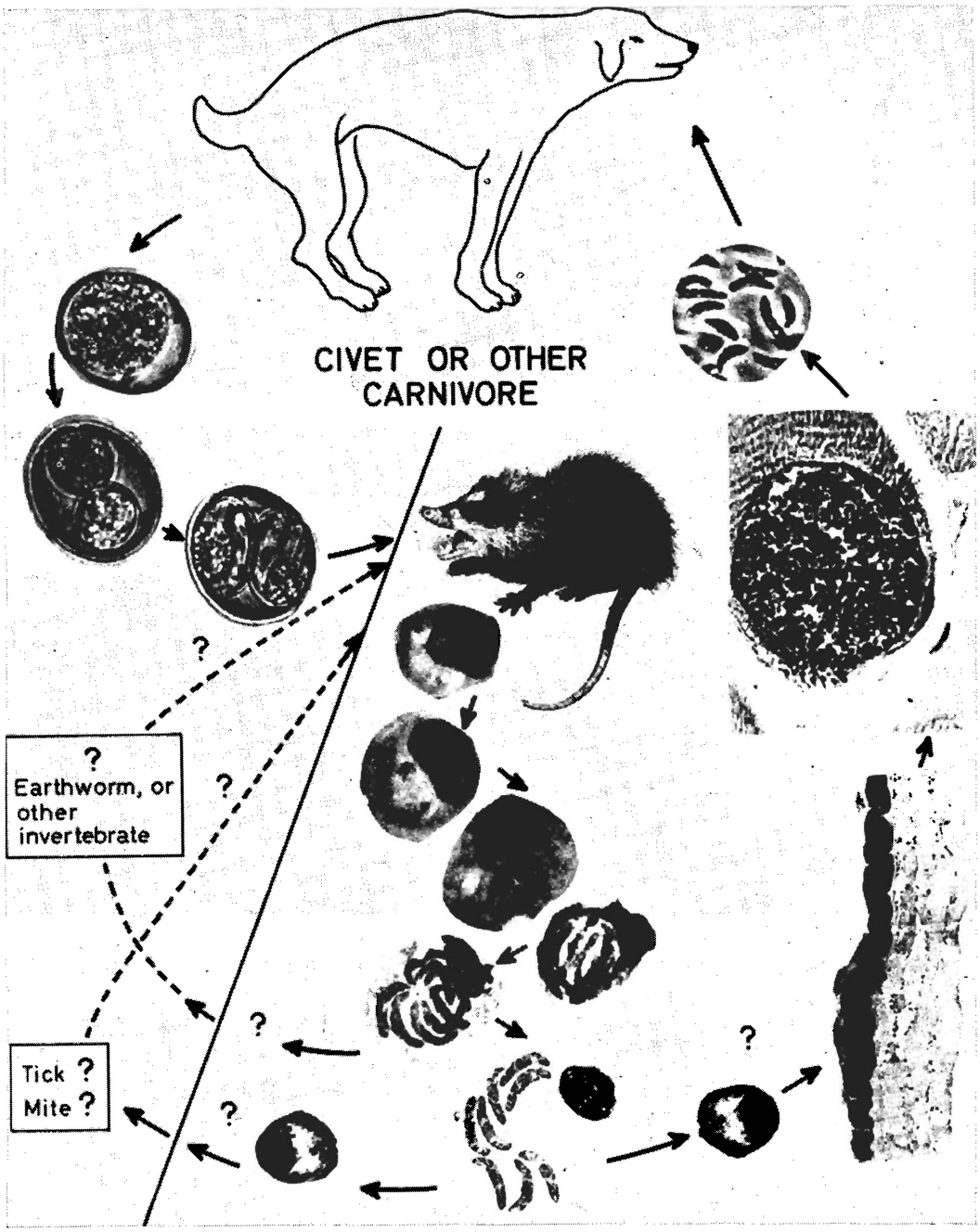


Fig. 34—Diagrammatic representation of life-cycle stages of *S. booliati* and *O. garnhami*.

1974). However, Lim Boo Liat (pers. comm.) states that the commonest predator and therefore the most likely definitive host of *Sarcocystis booliati* is the civet.

Species diagnosis of *Sarcocystis booliati* n.sp.

Type Host: *Echinosorex gymnurus*
Locality: Lowland forests, West Malaysia.
Cysts: Elongate and cylindroid with tapering ends; up to 4 mm long by 50-110 μm wide. Cyst wall smooth and very thin (less than 0.5 μm thick) with no trabeculae or cytophaneres. In skeletal but not in heart muscle.
Zoites: Small, thin and crescentic when fresh and more or less kidney-shaped when dry fixed, measuring 6.5 by 2.2 μm in smears.
Syntypes: In the Department of Medical Protozoology, London School of Hygiene and Tropical Medicine, Keppel Street, London W.C.1, England.

Species diagnosis of *Octoplasma garnhami* n.g., n.sp.

Type host: *Echinosorex gymnurus*
Locality: Lowland forests, West Malaysia.
Schizonts: Develop intracellularly in lymphoreticular cells, particularly in spleen.
Pseudocysts: Containing typically 8 zoites 13-17 μm by 2.5-4 μm; banana-shaped and with a distinctly staining nucleolus in dry fixed smears. Zoites mostly in monocytes of liver and spleen, dividing by binary or multiple fission to form the 8 zoites.
Other stages yet unknown.
Syntypes: In the Department of Medical Protozoology, London School of Hygiene and Tropical Medicine, Keppel Street London W.C.1, England.

SUMMARY

Sarcocystis booliati n.sp. is described from the moonrat *Echinosorex gymnurus* (Mammalia, Insectivora) from West Malaysia. The cysts are very thin-walled, not visible to the naked eye, and have no trabeculae or cytophaneres. They are found in skeletal but not heart muscle. The zoites are small, 5-8 by 2-3 μm with a mean of 6.5 by 2.2 μm, in dry fixed smears.

Octoplasma garnhami n.gen. n.sp., a parasite of undetermined taxonomic status but belonging to the Coccidiasina, Apicomplexa, is also described from the same host. Only schizonts and pseudocysts with typically 8 zoites, have so far been seen in monocytes of the spleen and liver. The zoites are large, 15 by 3 μm and have a distinct nucleolus even in dry-fixed smears.

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