

Three new species of gregarines from termites*

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Abstract. Three new species of gregarines, *Gregarina macrotermis* from *Macrotermis estherae* (Desn), *Steinina coptotermi* from *Coptotermes hzimi* and *Anthorhynchus hanumanthi* from *Odontotermes* sp. from India have been described and their systematic position is discussed along with that of all the gregarine parasites from termites.

Keywords. *Gregarina macrotermis*; *Steinina coptotermi*; *Anthorhynchus hanumanthi*; termite gregarines.

1. Introduction

Leidy (1881) described for the first time a gregarine parasite *Gregarina termitis* from the gut of the termite, *Termes flavipes*. The same gregarine was reported by Porter (1897) from 'some termites' (quoted from Henry 1933) and Ellis (1913) from *T. lucifagus*. Kirby (1927) described a second species, *G. mirotermis* from *Mirotermes panamensis* Snyder. Henry (1933) investigating the gregarine parasites of termites belonging to the genus *Zootermopsis* encountered many sporonts which were described earlier by Leidy (1881). He further observed that these sporonts occurred in multiple associations which is one of the distinguishing characters of the genus *Hirmocystis* Labbe. Hence he transferred it to the genus *Hirmocystis* under the name *H. termitis* (Leidy 1881) Henry 1933. He also described a new genus and species *Kofoidina ovata* from the termites *Zootermopsis nevadensis* and *Z. angusticollis* Hagen. Ghidini and Moriggi (1941) reported *G. ausoniae* from *Reticulotermes lucifugus* Rossi. Uttangi and Desai (1961) reported 3 species of *Hirmocystis*, *H. capritermes*, *H. indica* and *H. incola* all from *Capritermes incola* (Wasm) from India. Desai and Uttangi (1962 a, b) reported 2 more gregarines, *Sphaerocystis termitis* and *Actinocephalus capritermes* from the same host. They also described *Hirmocystis speculitermes*, *H. dharwarensis* and *Steinina termitis* from *Speculitermes cyclops sinhalensis* Roonwal and Sen Serma. Desportes (1963) described an aseptate gregarine, *Diplocystis zootermopsides* from *Z. nevadensis* Krohn. Gisler (1967) listed all the gregarine parasites reported from termites till 1967 and added 29 species. He, however, did not include in the list 4 species, *Kofoidina ovata* Henry 1933, *Hirmocystis capritermes* Uttangi and Desai (1962) *H. incola* Uttangi and Desai (1962) and *H. indica* Uttangi and Desai (1962). Further *G. termitis* Leidy 1881 and *Hirmocystis termitis* Henry 1933 are listed as two separate species while they are synonymous. Huger and Lenz (1976) described

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another unidentified coelomic gregarine from *Coptotermes acinaciformes*. Theodorides *et al* (1976) described two more species, *Gregarina darchenae* and *G. sp.* from *Cubitermes sp.* and *Basidentitermes potens* respectively.

Thus so far, 44 species of gregarines belonging to 6 genera, *Gregarina* Dufour, *Hirmocystis* Labbe, *Sphaerocystis* Leger, *Kofoidina* Henry, *Steinina* Leger and Duboscq and *Diplocystis* Schneider belonging to 5 families, Gregarinidae, Lecudinidae, Diplocystidae, Monocystidae and Actinocephalidae have been described from termite hosts.

In the following account 3 new species of gregarines, *Gregarina macrotermitis*, *Steinina coptotermi* and *Anthorhynchus hanumanthi* are described and their systematic position has been discussed.

2. Materials and methods

Macrotermes estherae (Desn) was collected from the earthen mounds; *Coptotermes heimi* was collected from the tree barks; and *Odontotermes sp.* was collected from the wet soil underneath the cashew-nut plants, all from the Andhra University at Waltair and surrounding areas. The termites after careful collection were brought to the laboratory, decapitated, dissected and examined under the low power of a compound microscope to detect infection. Observations on the fresh parasites were made by teasing out bits of infected gut in normal saline or Ringer's solution and examining under a compound microscope. Smears were wet-fixed either in Schaudinn's, alcoholic Bouin's or Carnoy's and stained with Delafield haematoxylin. Material for sectioning was fixed in alcoholic Bouin's, sectioned at $8\mu\text{m}$ thickness and stained with Heidenhain's iron haematoxylin. Cysts collected from the faecal matter as well as from the gut were kept either in a moist chamber or in 2.5% solution of potassium dichromate and examined at intervals for observing gametogenesis and sporogony. Smears of the cysts showing the gametes and spores were fixed and stained in the usual manner. The mucus envelope round the cysts was removed by rolling the cysts over a filter paper or with a pair of fine needles before fixation. Drawings were made with the aid of a camera lucida and measurements are given in microns.

3. Observations

3.1. *Gregarina macrotermitis* n. sp.

Host: *Macrotermes estherae* (Desn)

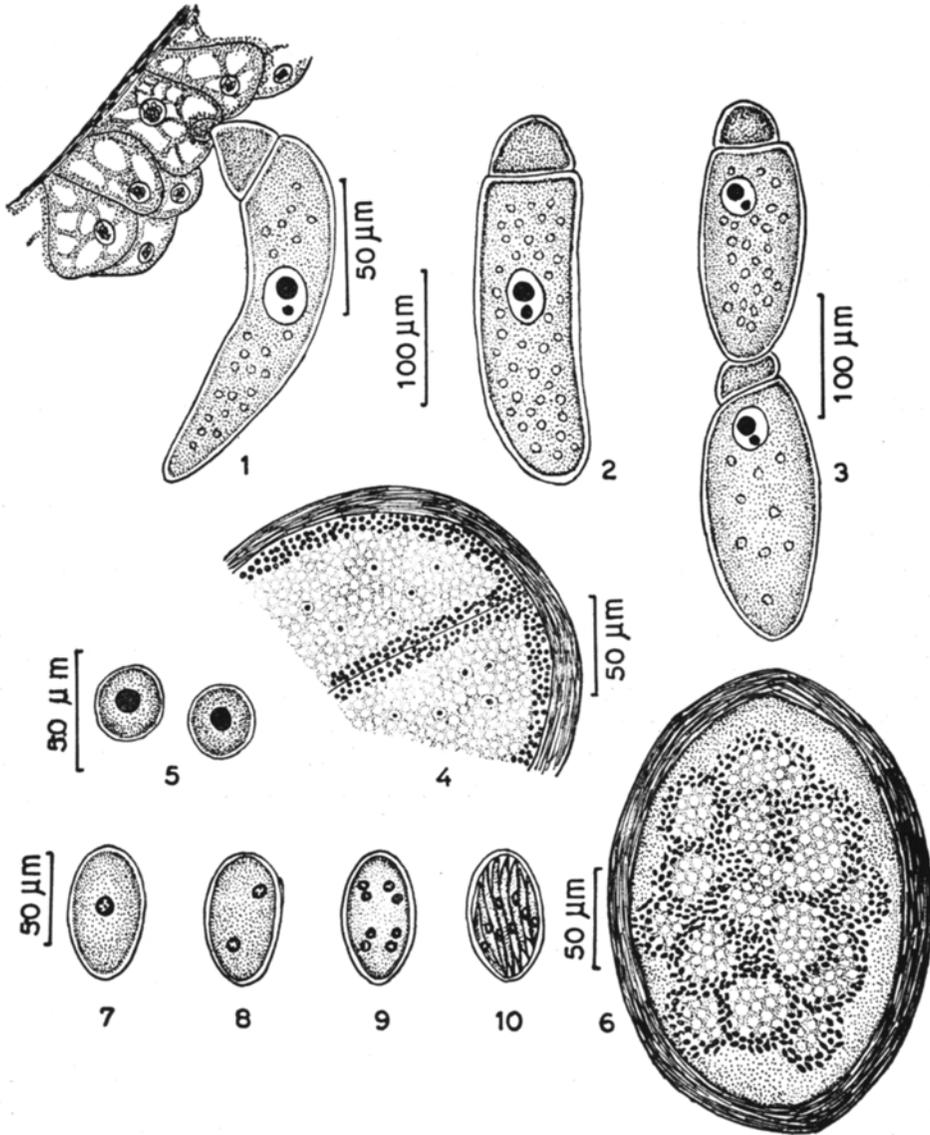
The foregut of 75% of workers collected from 24 different colonies were found infected with a gregarine parasite belonging to the genus *Gregarina* Dufour. Soldiers were not infected.

The earliest stage observed was in the lumen of the midgut attached to the epithelial cells. It was ovoidal and measured $26 \times 20\mu\text{m}$ with well differentiated epi-, proto- and deutomerites. The nucleus is vesicular with two deeply staining spherical endosomes of unequal sizes. The cytoplasm is alveolated and contains disc-like refringent bodies. Epimerite is knob-like and is completely embedded in the epithelial cell. The later stages in the growth of the parasite showed a rapid increase in the size of the deutomerite and the number of refringent granules (figure 1).

Sporonts measuring about $85.5 \times 25.0 \mu\text{m}$ were found free in the lumen and the epimerite is presumably left behind in the epithelial cell. The sporonts reached a maximum size of $200\text{--}220 \times 85\text{--}95 \mu\text{m}$ (figure 2). The sporadins exhibited two types of movement while they are in the lumen; one is the usual gliding movement and a second type where the parasite twists on itself and straightens out; during the process the posterior part is dragged forward to a distance equal to about $\frac{1}{4}\text{--}\frac{1}{2}$ the length of the parasite. The mature sporadins are biassociative (figure 3). They are common in the midgut. The primate and satellite are of equal size. Association stages found in the hindgut showed in addition to an end-to-end association a sideward attachment which are probably stages preceding cyst formation. Cysts are spherical or slightly oval ranging in size from $150\text{--}250 \mu\text{m}$ in diameter. An outer gelatinous ectocyst of $10 \mu\text{m}$ thickness protects the cysts from ciliate and bacterial attack. The cysts when passed out in the faecal matter are in a binucleate stage. Later the single nucleus in each gametocyte divided repeatedly by mitotic division resulting in the formation of several nuclei which are arranged along the periphery of the gametocytes in the final stage (figure 4). The gamete formation is completed in about 48 hr. They are isogamous. They are spherical in shape and measure $3.0\text{--}3.6 \mu\text{m}$ in diameter with a centrally placed nucleus (figure 5). Fertilisation between the gametes results in the formation of a zygote containing a synkaryon (figure 7). A well differentiated sporoblast wall is formed before the nuclear division is initiated. When the sporoblasts are formed the cytoplasm in the cyst is thrown into a number of folds or lobes and the sporoblasts are arranged along the surface of the folds. The cyst at this stage has a mulberry appearance (figure 6). The spores are oval measuring $7.2 \times 3.6 \mu\text{m}$ with a double wall. They are octozoic and the nuclei are arranged in two groups of four each at either pole (figures 8, 9 and 10). Sporogony is completed in about 5 days. The sporozoites are sickle-shaped and measure $6.0 \times 1.5 \mu\text{m}$ (figure 10).

3.1.1. *Discussion*: 44 species of gregarines are reported so far from termite hosts and 25 of them belong to the genus *Gregarina*. Kirby (1927) described *G. mirotermitis* basing his description on two stained biassociative stages and as has already been pointed out by Henry (1933) 'The measurements which are given are not strictly comparable to those given for other species'. Ghidini and Moriggi (1946) described *G. ausoniae* and they saw only a few association stages. Gisler (1967) described 32 species of *Gregarina* from 10 different genera of termites. They are 2 from *Thoracotermes macrothorax*, Sjostedt, 3 from *Cubitermes fungifaber* Sjostedt, 3 from *Cubitermes* sp., 1 from *Cucutermes* sp., 2 from *Anaplotermes* sp., 6 from *Proclubitermes* Sjostedt Von Rosen, 3 from *Allangnathotermes hypogeus* Silvestri, 1 from *Bellicositermes natalensis* Haviland, 1 from *Ophitermes grandilabius* Emerson and 1 from *Euchilotermes tensus* Silvestri. Huger and Lenz (1976) described another unidentified coelomic gregarine from *Coptotermes acinaciformes*. Theodorides *et al* (1976) described 2 more species, *Gregarina darchenae* and *G. sp.* from *Cubitermes* sp. and *Basidentitermes potens* respectively.

One of the important taxonomic characters of the genus *Gregarina* is the biassociative sporadins but in 10 out of the 23 species described by Gisler (1967) (Sp. No. 7, 8, 13, 14, 15, 16, 17, 18, 19 and 20) association stages have not been observed. He could assign a definite specific name only in 3 cases (table 1). In all other cases the description is incomplete and the species are designated by numbers only and hence



Figures 1-10. *Gregarina macrotermis* n. sp. 1. A fully grown trophozoite attached to the epithelial cell of the gut. 2. A sporont from the lumen of the gut. 3. Association stage. 4. A portion of a section of a multinucleate cyst showing the peripheral arrangement of nuclei in both the gametocytes. 5. Gametes. 6. A section of a cyst showing arrangement of spores. 7. Zygote. 8-9. Binucleate and octonucleate spores. 10. A spore showing the sporozoites.

a comparison is not possible. The gregarine described from the coelomic cavity of *Coptotermes acinaciformes* by Huger and Lenz (1976) is not identified. Theodorides *et al* (1976) described *Gregarina darchenae* from *Cubitermes* sp. (table 1) and a *Gregarina* sp. from *Basidentitermes potens* which is incompletely described and hence no comparison is possible with the present form.

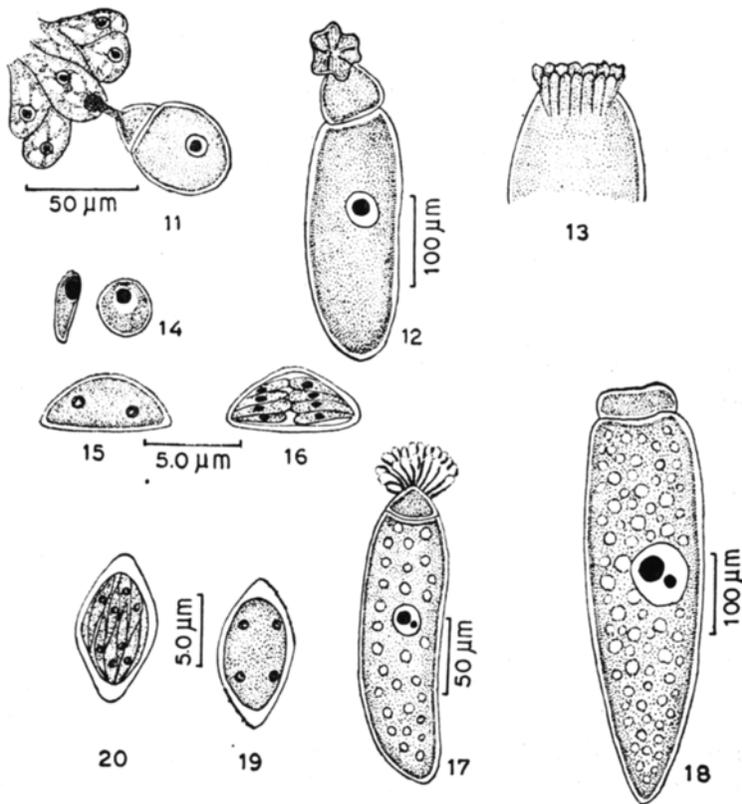
Table 1

Parasite	Host	Locality	Description
1. <i>G. mirotermitis</i> Kirby 1927	<i>Mirotermes</i> <i>panamensis</i>	Panama	Only 2 associative sporadins observed. $169 \times 39 \mu\text{m}$. Primate $94 \times 39 \mu\text{m}$, satellite $65 \times 27 \mu\text{m}$. Cysts and spores not described.
2. <i>G. ausoniae</i> Ghidini and Moriggi 1946	<i>Reticulotermes</i> <i>lucifugus</i> ; <i>Cubitermes</i> sp.	France	Characteristic association, Big ovoid primate, long conical satellite. Protomerite of primate hemispherical. Cysts and spores not described.
3. <i>G. thoracotermes</i> Gisler 1967	<i>Thoracotermes</i> <i>macrothorax</i> and <i>Echilotermes</i> <i>tensus</i> .	Germany	Association typical, $517 \times 28 \mu\text{m}$. Primate $150\text{-}267\cdot3 \times 15\text{-}20 \mu\text{m}$. Satellite $224\cdot5\text{-}250 \times 15\text{-}28 \mu\text{m}$. Protomerite of satellite has a cup-shaped depression into which fits forceps-like posterior part of primate. Protomerite of primate club-shaped. Cysts and spores not described.
4. <i>G. bancoi</i> Gisler 1967	<i>Thoracotermes</i> <i>macrothorax</i> , <i>Anaplotermes</i> sp. <i>Allangnathotermes</i> <i>hypogeus</i> and <i>Pericapritermis</i> <i>urgens</i> .	Germany	Association caudo-frontal. Association stages measure $434 \times 46 \mu\text{m}$; Primate $89\cdot2\text{-}301\cdot6 \times 20\cdot1\text{-}46\cdot0 \mu\text{m}$. Satellite $113\text{-}230 \times 30\text{-}46 \mu\text{m}$. Protomerite of primate dome-shaped Epimerite ball-shaped. A papilla like pit is sometimes seen at junction of epi- and proto-merite. Spores oval $4\cdot0 \times 7\cdot2 \mu\text{m}$.
5. <i>G. cucumiformis</i>	<i>Allangnathotermes</i> <i>hypogeus</i>	Germany	Association stages $527 \times 27 \mu\text{m}$. Primate $262\cdot6 \times 23\cdot2$. Epimerite irregular, more or less ball-shaped. Protomerite of primate cylindrical Deutomerite suddenly tapers in the middle and forms thin tail. Posterior part of the primate forms a conical piston like structure which passes through the septum and enters the cytoplasm of the protomerite of satellite.
6. <i>G. darchenae</i> Theodorides <i>et al</i> 1976	<i>Cubitermes</i> sp.	France	Association caudofrontal, $330 \mu\text{m}$. Primate and satellite have same dimensions. Terminal part of the deutomerite of primate deeply embedded in a cup-like structure in the protomerite of satellite.
7. <i>G. macrotermitis</i> n. sp. (Present paper)	<i>Macrotermes</i> <i>estherae</i> Desn	India	Association caudofrontal, $450\text{-}500 \times 85\text{-}95 \mu\text{m}$. Primate and satellite of approximately same dimensions Epimerite knob-like. Cysts spherical $150\text{-}250 \mu\text{m}$. Gametes isogamous, $3\cdot0\text{-}3\cdot6 \mu\text{m}$. Spores oval $7\cdot2 \times 3\cdot6 \mu\text{m}$. Sporozoites sickle-shaped $6\cdot0 \times 1\cdot5 \mu\text{m}$.

Hence the present form is compared with the 6 completely described species, *G. mirotermitis*, *G. ausoniae*, *G. bancoi*, *G. thoracotermis*, *G. cucumiformis* and *G. darchenae* (table 1). It is clear that the present form does not fully agree with any of the species so far described. The spores are described in the case of *G. bancoi* alone and they measure $4\cdot0 \times 7\cdot2 \mu\text{m}$ while in the present form they measure $6\cdot0 \times 1\cdot5 \mu\text{m}$. Further the present parasite is reported from a different host and different geographical locality and as such is considered a new species and the name *Gregarina macrotermitis* n. sp. is proposed for the same.

3.2. *Steinina coptotermi* n. sp.Host: *Coptotermes heimi*

About 2% of the workers examined showed infection. The trophozoites were found in the midgut. The smallest trophozoite encountered was about $50 \times 30 \mu\text{m}$ with spherical deutomerite. The epimerite is a button-like structure at the tip of a small neck and is embedded in the epithelial cell (figure 17). The nucleus is vesicular with a single deeply stained endosome. Some of the cephalonts were found free in the lumen of the midgut. They attain a maximum size of $150 \times 70 \mu\text{m}$. The epimerite in such forms is found changing its shape into a flattened structure with wavy margins (figures 12 and 13). Sporadins are solitary varying in size from $350\text{--}450 \times 50\text{--}150 \mu\text{m}$ with a long deutomerite and a short protomerite. The cytoplasm is finely alveolated and showed a few refringent granules. The cysts are spherical in shape measuring about $600\text{--}800 \mu\text{m}$ in diameter with an outer thin gelatinous ectocyst. The cysts collected from the midgut and kept in a moist chamber showed the formation of the gametes in about 1 hr. Gametes anisogamous, macrogametes spherical with



Figures 11-20. 11-16. *Steinina coptotermi* n. sp. 11. A cephalont attached to the gut wall (note the knob like epimerite). 12. A cephalont with expanded epimerite. 13. An enlarged view of the epimerite (side view). 14. Gametes. 15. A binucleate spore. 16. A spore showing the sporozoites. 17-20. *Anthoshynchus hanumanthi* n. sp. 17. A cephalont. 18. A sporont. 19. A tetranucleate spore. 20. A spore showing sporozoites.

centrally placed spherical nucleus containing an eccentrically placed endosome. The male gametes are elongated with a deeply stained nucleus located at one end of the body. No locomotor organelle has been observed (figure 14). The cysts collected from the faecal matter showed the fully formed spores. The spores are arranged along the periphery leaving a central mass of residual protoplasm. The spores are hat-shaped measuring about $8.0 \times 3.2 \mu\text{m}$ and are octozoic (figures 15 and 16).

3.2.1. *Discussion*: The structure of the epimerite and the presence of solitary sporonts qualify the inclusion of the present form in the genus *Steinina* Leger and Dubosq (Fam: Actinocephalidae). So far only 3 species of *Steinina* are described from termite hosts of which only one *S. termitis* from *Speculitermes cyclops sinhalensis* has been completely described (Uttangi and Desai 1962). The other two, *Steinina* sp. 1 and *Steinina* sp. 2 described by Gisler (1967) are incompletely described. The present form differs from *S. termitis* in having solitary sporonts and hat-shaped spores. The epimerite in *S. termitis* is a globular body with a transparent sap changing its shape into a digitiform process to a disc-like body whereas in the present form it is a small button-like body placed at the tip of a short neck which can change its shape into a plate with wavy margin. In view of the differences mentioned and also because this is the first report from *Coptotermes heimi* it is considered new to science for which the name *Steinina coptotermi* n. sp. after the host is proposed.

3.3. *Anthorhynchus hanumanthi* n. sp.

Host: *Odontotermes* sp.

Although a number of termites from different localities in the University Campus were examined we came across the infection only on two occasions. Both the intensity and percentage infection were low. The trophozoites appeared as opaque white bodies in the midgut. They are elongated with a long deutomerite and a small protomerite. They varied in size from $150\text{--}250 \times 60\text{--}80 \mu\text{m}$. The nucleus is vesicular with two endosomes of unequal size. The cytoplasm is hyaline and is almost filled with refringent granules. The epimerite is a large flattened fluted disc with transparent edges (figure 17). The sporadins are solitary and are seen in the lumen of the gut. They measure $30\text{--}0600 \mu\text{m}$ with a long deutomerite, the posterior end of which is bluntly pointed. The protomerite is rectangular in shape with a shallow depression at the anterior end (figure 18). Cysts were rarely seen in the faecal matter and when encountered were filled with spores. The cysts are oval in shape measuring about $350\text{--}500 \times 600\text{--}800 \mu\text{m}$ in diameter. They have a thick cyst wall but an ectocyst is absent. Spores are biconical with polar thickenings measuring about $10\text{--}12 \times 5.0\text{--}5.6 \mu\text{m}$ (figure 19). Spores are octozoic and the sporozoites are spindle shaped (figure 20).

3.3.1. *Discussion*: The genus *Anthorhynchus* is characterised by a large flattened fluted disk-like epimerite and biconical spores which are chained laterally. The present form shows all these features and thus qualifies for inclusion in this genus. The only species described is from an arachnid, *Phalangium opilio* (Arachnida: Phalangidae). It differs from the present form considerably in its measurements. The sporadins in the present form reach a maximum length of $600 \mu\text{m}$ whereas in

A. sophiae they reach a maximum length of 2 mm, the epimerite alone measuring 200 μm . The spores in the present form are biconical with polar thickenings varying in size from 10.0–12.0 \times 5.0–6.5 μm and are chained laterally where as in *A. sophiae* they are biconical and measure 7.0 \times 5.0 μm . In view of the above differences and the difference in the host we consider at a new species for which the name *A. hanumanthi* n. sp. is proposed.

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