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ORIGINAL ARTICLE

A new species of *Quadruspinospora* Sarkar and Chakravarty, 1969 (Apicomplexa:Conoidasida) from Orthopteran Insects of Manipur, India

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Abstract The description of a new species of cephaline gregarine *Quadruspinospora oxyae* sp.nov from a grasshopper (Orthoptera:Acrididae) is presented. Trophozoite has an elongated body measuring 120–163.6 (146 ± 14.1) µm with digitiform epimerite that is in the form of a question mark, measuring 6.5–23.6 (13.8 ± 5.3) µm in average. The mature sporadins associate sidewise in pairs and move together. Spherical Gametocyst measuring 90–129.7 (116 ± 11.1) µm × 59.7–97.4 (74.4 ± 13.4) µm in dimensions are present. Oocysts are oval, measuring 7.2 × 11.5 µm in length. Length of spores 19.5–21.5 µm, provided with four long spines, two at each pole, which is a characteristic of the genus *Quadruspinospora* Sarkar et Chakravarty, 1969.

Keywords *Quadruspinospora* · Cephaline gregarine · Apicomplexa · Conoidasida

Introduction

The present paper records the description of new species of septate gregarines (Apicomplexa: Conoidasida) from the midgut of a grasshopper (Orthoptera; Acrididae) of Manipur, India. Sarkar and Chakravarty (1969) established the genus *Quadruspinospora* to incorporate the cephaline gregarines inhabiting the various parts of the mid gut of the grasshopper. They characterized the genus as having solitary, elongated trophozoite with a sub-spherical epimerite having 8–12 stumpy digitiform processes, hemispherical protomerite and granular deutomerite which is broadest immediately behind the septum; spherical nucleus with several karyosomes; spherical, thick-walled gametocysts dechiscing by simple rupture; oval spores with a pair of very long spines at each pole and intracellular development. Chakraborty and Haldar (1974) observed that different species of grasshoppers were infested with the cephaline gregarines belonging to the genus *Quadruspinospora*. Later, Haldar and Chakraborty (1975) redefined the genus *Quadruspinospora* by solitary, elongated trophozoite with a sub-spherical epimerite having a variable number of stumpy, digitiform processes; hemispherical protomerite and granular deutomerite, broadest immediately behind the septum, spherical, subspherical or elliptical nucleus in the adult trophozoite; thick-walled, spherical gametocysts dehiscing by simple rupture; oval spores with four very long spines; two at each pole and intracellular development, confined to the epithelial cells of the hepatic caeca. Subsequently a number of new species were added to this genus from different Orthopteran species by Haldar and his collaborators, all with four polar spines in their oocysts but having epimerites with highly flexible structures, even without any digitiform process (Chakraborty and Haldar 1974; Haldar and Chakraborty 1975, 1976, 1978; Kundu and Haldar 1983; Datta et al. 1990). Epimerites might be short and cone like either a simple knob or cauliflower-like without any digitiform process; gamonts solitary and spherical gametocysts that dehisced by a simple rupture releasing ovoid oocysts having four typical spines, two at each pole (Biplob et al. 2008). The description of the new

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species *Quadruspinospora oxyae* sp.nov supported with photomicgraphs are provided (Fig. 1).

Materials and methods

The Adults of *Oxya hyla hyla* collected from various grass fields of Manipur (24°44′N, 93°58′E) in the morning between 6 and 8 a.m. with the help of net, were kept in glass tubes and brought alive to the laboratory for investigation from April, 2011 to December, 2014. These were decapitated, their guts carefully dissected out under a dissecting microscope and gently pressed to expel the parasites from the gut lumen. Thin smear preparations were fixed in Schaudinn's fixative and subsequently stained with Heidenhain's haematoxylin (Kudo 1966). Gametocysts were recovered from the hind gut and placed in moist chambers (>80 % relative humidity) for sporulation (Sprague 1941). The structure of the oocysts were studied by using Lugol's iodine solution. Figures of stained specimens were drawn with the aid of a camera lucida. Measurements of fresh materials were taken using an ocular micrometer calibrated with a stage micrometer. All measurements, unless other-wise mentioned were in micrometers. Twenty specimens each of mature gamonts and associations were randomly meas-ured from the infected hosts. Similarly, twenty gametocysts and twenty individual oocysts were measured. Measurements were taken from widest part of protomerite, deutomerite, nucleus, gametocyst and oocyst and presented in this paper as range values, followed by means, standard er-rors and sample sizes in parentheses. Blue filters were used for measurements and daylight filters were used for observation of colour in living specimens. Nomenclature for shapes used in this manuscript conforms to those of Clopton (2004) (Fig. 2; Table 1).

Abbreviation

The following abbreviations are used: LD = length of deutomerite, LE = length of epimerite, LN = length of nucleus, LP = length of protomerite, TL = total length,

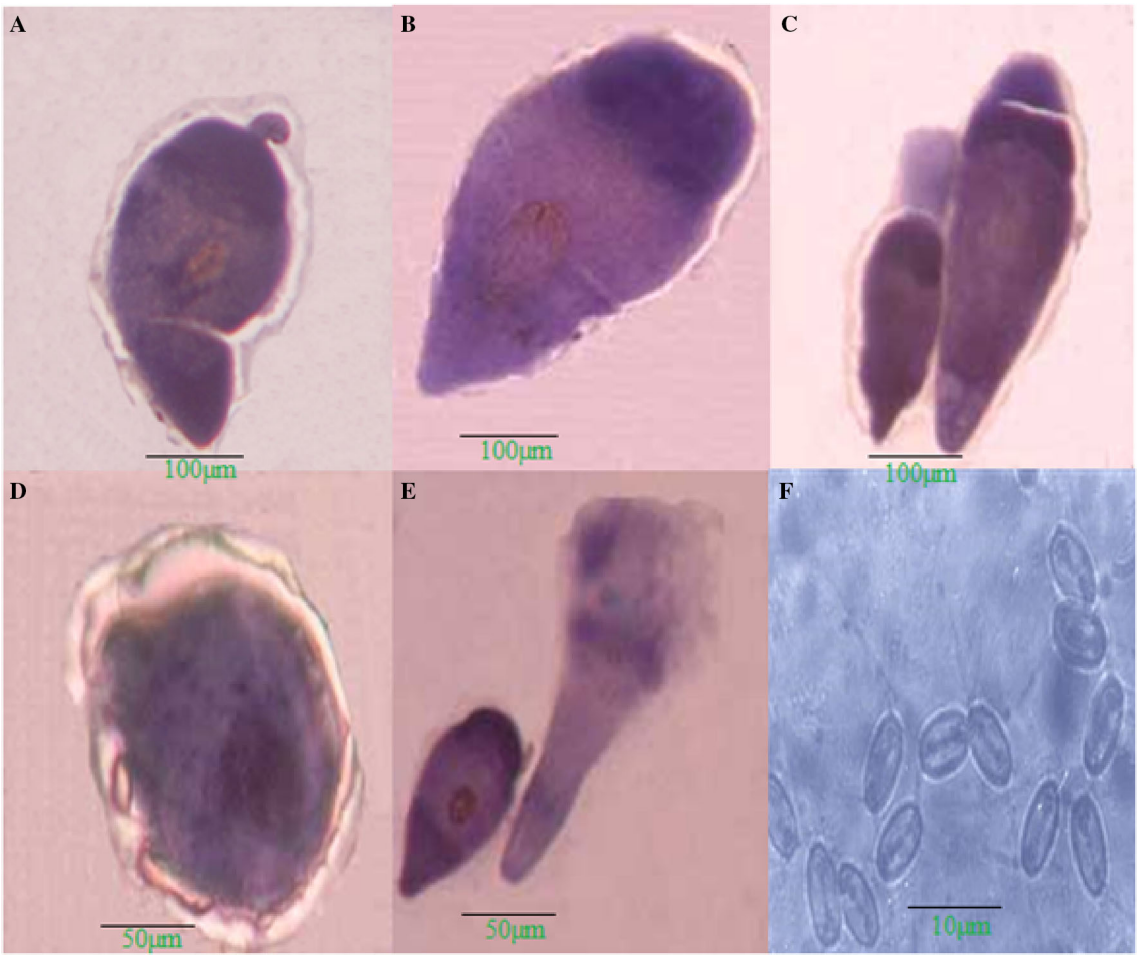

Fig. 1 **a** A mature trophozoite, **b** young trophozoite, **c** sporadin in syzygy, **d** gametocyst, **e** a young sporadins showing an anchor shape and a typical flower vase like structure and **f** spore with

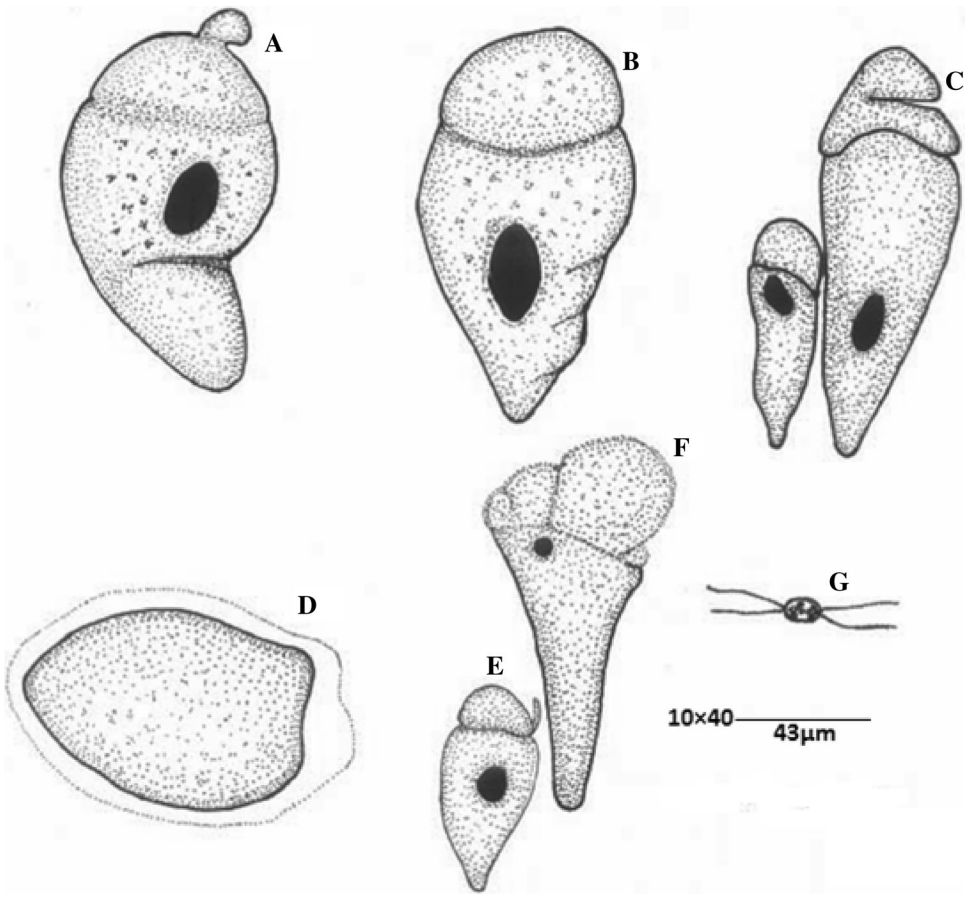

Fig. 2 **a** Mature Trophozoite, **b** Young Sporadin, **c** Sporadin in Syzygy, **d** Gametocyst, **e** a young Sporadins showing an anchor shape, **f** a typical structure flower vase like, **g** Spore with spines

Table 1 Showing R,$\overline{X}$, SD, SE and CV% of measurement of *Quadruspinospora oxyae* sp. nov

Different parts	R	$\overline{X}$	SD	SE	CV%
TL	120–163.6	146.0	14.14	3.16	9.68
LE	6.5–23.6	13.8	5.3	1.1	38.40
LP	27.5–47.1	37.4	5.9	1.3	15.77
WP	40.1–92.9	78.8	12.9	2.8	16.37
LD	87.9–116.5	96.9	8.5	1.9	8.77
WD	66.8–106.6	91.1	11.4	2.5	12.5
LN	20.5–30.4	25.1	2.7	0.6	10.75

WD = width of deutomerite, WP = width of protomerite. The ratios used are the ratio of the length of protomerite to total length (LP:TL) and the ratio of the width of protomerite to the width of deutomerite (WP:WD).

Results

Trophozoite The fully grown trophozoite has an elongated body measuring 120–163.6 (146 ± 14.1) in total length. Petaloid epimerite with stalk measuring 6.5–23.6 (13.8 ± 5.3) µm. The protomerite is hemispherical and broader than long, measures 27.5–47.1 (37.4 ± 5.9) µm × 40.1–92.9 (78.8 ± 12.9) µm. A thick septum separates the protomerite from the deutomerite. The deutomerite is cylindro–conical with tapering posterior extremity and is broadest just behind the septum. It measures 87.9–116.5 (96.9 ± 8.5) µm × 66.87–106.6 (91.1 ± 11.4) µm in average dimensions. The cytoplasm is granular. The pellicle is moderately thick but quite flexible. The nucleus is spherical and ovoidal with distinct nuclear membrane and several chromatin granules measuring 20.5–30.4 (25.1 ± 2.7) µm in diameter.

Sporadins The sporadins are characteristically solitary, 131.4–169.6 (142 ± 8.5) µm in total length. The pellicle is exceedingly thick. The protomerite is oblong or hemispherical in shape and measures 25.9–48.9 (38.3 ± 6.2) µm × 42.3–87.3 (72.4 ± 13.9) µm. in average dimensions. The deutomerite is cylindro-conical in shape with a pointed posterior extremity. It measures 110.9–145.6 (131 ± 10.5) µm × 39.9–89.3 (74.3 ± 15.5) µm in average. The nucleus is spherical in shape and measures 19.5–33.6 (27.3 ± 4.4) µm in diameter. The cytoplasm is packed with numerous coarse granules.

Association The mature sporadins associate sidewise in pairs and move together for some time. Ultimately these enclose themselves within a common cyst wall. A thick gelatinous ectocyst surrounds the enclosed gametocytes.

Gametocyst Almost spherical measuring 90–129.7 (116 ± 11.1) μm × 59.7–97.4 (74.4 ± 13.4) μm. The gametocyst dehisces by simple rupture at about 48 h of development releasing the spore.

Spores The spores are oval in shape measuring 11.5 × 7.2 μm average in dimension. The spores are provided with four long spines, two at each pole. Eight globular sporozoites are seen, four at each pole, with maturation of the oocyst.

Taxonomy summary

Type material	*Quadruspinospora oxyae* sp.nov.
Type host	*Oxya hyla hyla* (Order: Orthoptera).
Type locality	Canchipur Imphal- west.
Site of infection	MID GUT.
Prevalance	39 out of 60 (65 %).
Paratype	MU/0210/14, deposited in the Protozoan Collection of Parasitology Section, Centre of Advanced Studies in Life Sciences, Manipur University, Canchipur-795003, India. Another Paratype deposited in the National Zoological Collection (Accession no. Pt. 3026) of the Zoological Survey of India, Kolkata.
Holotype	MU/019/14, deposited in the Protozoan Collection of Parasitology Section, Centre of Advanced Studies in Life Sciences, Manipur University, Canchipur-795003, India.

Measurements The summary of measurements in micrometers of preserved (fixed and stained) Trophozoites and Sporadins are given below:

Paratype: (20)

Trophozoite

TL = 120–163.6 (146 ± 14.1)
LE = 6.5–23.6 (13.8 ± 5.3)
LP = 27.5–47.1 (37.4 ± 5.9)
LD = 87.9–116.5 (96.9 ± 8.5)
LN = 20.5–30.4 (25.1 ± 2.7)
WP = 40.1–92.9 (78.8 ± 12.9)
WD = 66.87–106.6 (91.1 ± 11.4)
LP: LT = 1: 3.5
WP: WD = 1: 1.2

Sporadin

TL = 131.4–169.6 (142 ± 8.5)
LP = 25.9–48.9 (38.3 ± 6.2)
LD = 110.9–145.6 (131 ± 10.5)
LN = 19.5–33.6 (27.3 ± 4.4)
WP = 42.3–87.3 (72.4 ± 13.9)
WD = 39.9–89.3 (74.3 ± 15.5)
LP: TL = 1:5.0
WP: WD = 1:1.0

Holotype

Trophozoite

TL = 129
LE = 11.25
LP = 32.25
LD = 96.75
LN = 22.5
WP = 43.0
WD = 69.87

Sporadin

TL = 134.3
LP = 26.8
LD = 111.5
LN = 21.5
WP = 43
WD = 41.3

Discussion

Presence of epimerite petaloid with stalk, solitary sporadins, dehiscence of cyst by simple rupture and spherical spores with spines confirm the inclusion of this gregarine under the genus *Quadruspinospora* Sarkar and Chakravarty, 1969 (Table 2).

The present form closely resembles *Q. chakravartyei*, Chakraborty and Haldar, 1976 in the ratio of WP: WD; however they differ in the measurements, shape of the epimerite, gametocyst and in host, (Epimerite 20–24 μm digitiform process gametocyst 350 × 420 μm and found in *spathoslernum* sp for *Q. chakravartyei*). The host is same with that of *Q. aleopii*, Sarkar and Chakraborty, 1969 and *Q. acridii,* Haldar and Chakraborty, 1976. The present form differ widely in the shape of the epimerite, nucleus and the other measurements including the general body

Table 2 Showing the comparative characters of *Q. aleopii, Q. indoaiolopii, Q. acridii* and *Quadruspinospora oxyae* sp. nov

Characters	*Q. aleopii* Sarkar and Chakraborty, 1969	*Q. chakravartyei* Chakraborty and Haldar, 1976	*Q. acridii* Haldar and Chakraborty, 1976	*Quadruspinospora oxyae* sp. nov
Total length	441.5 μm	52.5–457.5	106.3–533.1 μm	120–163.6 μm
Epimerite	Numerous short, stumpy brush—like digitiform processes	Width 20–24 Digitiform processes	Knob- like with nine digitiform processes	Question mark like Digitiform process
Protomerite	Hemispherical	Dome-shaped	Hemispherical	Hemispherical
Nucleus	Oval	Spherical to elliptical	Ovoidal	Spherical
Gametocyst	Spherical, 420 μm; equal or unequal gametocytes	350.0 × 420.0 μm; equal or unequal gametocytes	383.2–497.0 × 233.2–297.9 μm; equal or unequal gametocytes	90–129.7 × 59.7–97.4 μm, spherical gametocyte
Spore	Oval; 8.3 × 5.0 μm; length of the spine 24.9–33.2 μm	9.0 × 5.0 μm; length of each spine 30.0 μm	Oval; 6.6 × 5.0 μm; length of the spine 18.3–28.2 μm	Oval; 11.5 × 7.2 μm, length of the spine 19.5–21.5 μm, provided with four long spines, two at each pole
LP:LT	1:2.0–10.0 (7.9)	1:6.1	1:2.7–9.3 (5.8)	1:3.5
WP:WD	1:0.4–2.0 (1.06)	1:1.2	1:08–1.4 (1.2)	1:1.2
Host	*Oxya hyla* Serville	*Spathoslernum* sp.	*Oxya* sp.	*Oxyla hyla hyla*
Locality	Naihati	Kalyani	Jalpaiguri	Manipur, Imphal

shape from that of the above mentioned species (Epimerite stumpy brush like digitiform process, nucleus oval, gametocyst 420 μm, spore length 8.3 × 5 μm, spine 24.9–33.2 μm in *Q. aleopii*. Epimerite knob-like with nine digitiform process, nucleus oval, gametocyst 383.2–947.0 × 233.2–297.9 μm, ovoidal, spore 6.6 × 5 μm, spine 18.3–28.2 μm in *Q. acridii*). It, appears that the gregarines of the present form is quite different from of the species described so far in general shape and morphometrical values. As, such, the present gregarines is designated as *Quadruspinospora oxyae* sp.nov. and proposed as a species new to science.

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