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Ultrastructure and prevalence of the septate gregarine *Quadruspinospora indoaiolopii* haldar and chakraborty, 1976 (Apicomplexa: Eugregarinida: Conoidasida), parasitic in the alimentary canal of the grasshopper *aiolopus thalassinus tamulus* fabricius, 1798 (Orthoptera: Acrididae)

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Abstract
Septate gregarines (Apicomplexa: Eugregarinida: Conoidasida) are the most varied and common protozoan parasites of invertebrates, especially arthropods. The genus *Quadruspinospora* has sixteen species, all from orthopterans and all from India except *Q. mexicana*. *Quadruspinospora indoaiolopii*, commonly found in the midgut of *Aiolopus thalassinus tamulus* (Orthoptera: Acrididae). There have been no ultrastructural studies of any *Quadruspinospora* species reported from India. The present study aims to investigate the surface morphology of *Q. indoaiolopii* by scanning electron microscopy. For scanning electron microscopy, mature trophozoites and gamonts were isolated and fixed in 2.5% glutaraldehyde solution with Na-cacodylate buffer (pH 7.4). 29 grasshoppers out of 124 were found to be infected with *Q. indoaiolopii*. During the monsoon and early winter, prevalence remains higher, averaging 43.3%. Epimerite is subspherical and knob-shaped, with 10–23 digitiform processes. Gamonts are elongated and solitary. Gametocytes are almost orbicular and dehisce by simple rupture. Oocysts are elliptoid in shape and armed with four long spines; each pole has two. Under SEM, the epicytic folds look like ridges that run the whole length of the trophozoite and gamont. Both protomerite and deutomerite remain covered with longitudinal, parallel epicytic folds, which are undulating in appearance. Epicytic folds are absent in the epimerite region. The epimerite-protomerite junction has a short, narrow neck-like structure, and the deutomerite exhibits deep longitudinal grooves. The density of folds is about 7–9 epicytic folds per micron, compared to *Q. mexicana*, which had five folds per micron.

Keywords: apicomplexa; *Quadruspinospora indoaiolopii*; *Aiolopus thalassinus tamulus;* septate gregarines

Introduction
The short-horned grasshopper, *Aiolopus thalassinus tamulus* (Fabricius, 1798), is commonly found in grasslands as well as crop areas such as rice, wheat, and vegetable farms all over India and does significant harm to agricultural crops (Mandai *et al*., 2007) [7]. Grasshoppers in West Bengal were frequently reported to have a septate gregarine infection. In the phylum Apicomplexa, septate gregarines are one of the most diverse and common species of protozoan parasites (Smith and Clopton, 2003) [14], which primarily affect invertebrates (Clopton, 2002) [2]. Arthropods have the highest diversity of septate gregarine among invertebrates, and insects are their preferred hosts. Grasshoppers belonging to the genus *Aiolopus* were found to be infected with four species of septate gregarines: *Quadruspinospora aelopii* (Sarkar and Chakraborty, 1969) [12], *Q. indoaiolopii* (Haldar and Chakraborty, 1976) [4], *Phleobum cloptoni* (Chatterjee and Haldar, 2003) [1], and *P. janovii* (Patra and Haldar, 2004) [10].

The genus *Quadruspinospora* was established by Sarkar and Chakravarty (1969) [12] from the midgut of the short-horned grasshopper, *Aiolopus* sp. in which the trophozoites are solitary and elongated. The epimerite in younger trophozoites is conical or knob-like. In mature trophozoites, the epimerite is subspherical, often with a few stumpy digitiform processes or even none at all. The gametocyst is almost orbicular in shape and has a pronounced ectocyst; the cyst dehisces with a simple rupture. Oocysts are ellipsoid-shaped and have two long spines at each pole. There are currently sixteen recognised species in the genus *Quadruspinospora*, *Q. aelopii* Sarkar & Chakravarty, 1969; *Q. chakravartyei* Chakraborty & Haldar, 1975; *Q. indoaiolopii* Haldar & Chakraborty, 1976; *Q. acridaii* Haldar & Chakraborty, 1976; *Q. megaspinosa* Haldar & Chakraborty, 1976; *Q. atractomorphii* Haldar & Chakraborty, 1978; *Q. dichotoma* Kundu & Haldar, 1983; *Q. platyepimerita* Datta, Gosh & Haldar, 1990; *Q. adigitalis* Datta, Gosh & Haldar, 1990; *Q. gesonulae* Datta, Gosh & Haldar, 1990; *Q. jalpaiguriensis* Datta, Gosh & Haldar, 1990; *Q. hieroglyphae* Mandal & Ray, 2007; *Q. cloptoni* Modak, Basu & Haldar, 2008; *Q. caudata* Modak, Basu & Haldar, 2008; Q. *oxyae* Yumnam & Mohilal, 2015; Q. *mexicana* Medina-Duran *et al*., 2020. They are all reported from various orthopteran species, and except for Q. *mexicana* (Medina-Durán *et al.*, 2019) [8], all are reported from India.

There have been no ultrastructural studies of any *Quadruspinospora* species reported from India. In the current study we aim to investigate the surface morphology of both tropozoite and gamont stage of *Q. indoaiolopii* Haldar and Chakraborty, 1976 by scanning electron microscopy.

Materials and methods
Adult host grasshoppers (*A. thalassinus tamulus*) were collected from the agricultural fields, grasslands, and margins of the Sal forests of Bankura district (23°20'49.4"N 87°14'09.8"E, 23°18'59.6"N 87°12'25.9"E, and 23°17'58.6"N 87°11'51.1"E). From July 2021 to June 2022,

collections were made twice a month in the morning. The collection was done by the insect collection net.

For the examination of septate gregarine infections, adult grasshoppers were brought alive in the laboratory. Host grasshoppers were decapitated before being dissected, and following dissection, fine forceps were used to remove each host's alimentary canal. The guts of the host arthropods were placed on a glass slide with a drop of physiological saline, and the gregarines were expelled from the gut lumen using two fine needles. Different lifecycle stages of the septate gregarines were isolated with a fine brush, and microphotographic images of live specimens were taken with a Zeiss Axioscope.A1 differential interphase contrast (DIC) microscope. The gametocysts were kept in a moist chamber on depression slides for sporulation (Sprague 1941) [15]. To analyse the oocyst structure, a solution of Lugol's iodine was applied. It is preferred to name the shapes using Clopton's nomenclature of plane shapes (Clopton, 2004) [3]. Septate gregarines were identified to species level using Haldar and Chakraborty (1976) [4].

For scanning electron microscopy, mature trophozoites and gamonts were isolated with a fine brush and washed in physiological saline for at least three times. The septate gregarines were fixed for 2 hours at room temperature using a 2.5% glutaraldehyde solution with Na-cacodylate buffer (pH 7.4). Samples were dehydrated using a graduated alcohol series, and subsequently, they were critical point dried using CO2. Individual gregarine samples will be mounted on stubs, 5 nm gold-palladium will be sputtered onto them, and they will then be viewed under a SEM.

Results

Sixty-four individuals have shown the occurrence of septate gregarine in their gastrointestinal tract, and twenty-nine individuals were found to be infected with *Q. indoaiolopii*, out of a total of one hundred and twenty-four *A. thalassinus tamulus.*

Light microscopic description of *Q. indoaiolopii*

Trophozoite (Fig. 1A): At the earliest stage of *Q. indoaiolopii*, there is a broadly elliptoid or orbicular body that has a prominent nucleus and develops into the epithelial cell that makes up the inner layer of the midgut. Later on, the protomerite and the deutomerite become more noticeable, and an epimerite with a limited number of digitiform processes forms in front of the protomerite, allowing the latter to stay connected to the epithelial cell in a dangling form in the lumen. At maturity, a trophozoite develops into a solitary, elongated, and segmented form. The distal end of the subspherical knob-shaped epimerite has 10–23 digitiform processes, which are fired from the end. The protomerite more or less very shallowly ovoid in shape, measuring 21.2-96.5 µm X 38.1- 205.4 µm. Deutomerite is very deeply obdeltoid in shape, measuring 76.4-470.1 µm X 35.4- 278.7 µm. The pellicle is uniformly thick and thickest at the anterior end of the protomerite. The nucleus might be orbicular, very broadly elliptoid, or broadly elliptoid in shape.

Gamont (Figs. 1B and C): Gamonts are elongated and solitary as well, although epimerite is not present. Protomerite and deutomerite are comparable to trophozoites in appearance. Pellicle is likewise thick, and the thickness of the pellicle is significantly larger in the anterior end. No association was observed.

Gametocyst (Fig. 1D): Milky white, almost orbicular gametocyst measuring 245 to 465 µm with two identical gametocytes encased within a conspicuous, thin ectocyst. Once the gametocyst has developed for around 72 hours inside the moist chamber, it dehisces by simple rupture.

Oocyst (Fig. 1E): Oocysts are elliptoid in shape and measure 10 µm × 5 µm; they are equipped with four long spines, each pole has two, each reaching 40 µm in length, and their sizes and shapes are quite consistent.

Scanning electron microscopic description of *Q. indoaiolopii*

Trophozoite (Figs. 2A and B): The trophozoite stage of the parasite's life cycle is characterised by the presence of many longitudinal folds on the surface of the cell (Fig. 2A). These folds aid in determining the shape of the cell and are organised as generating lines along the surface. The epicytic folds may be seen as ridges running the whole length of the body. The folds have an undulating appearance. There is no evidence of epicytic folds in the epimerite region. (Fig. 2B). The protomerite has the shape of a dome, and the front section of it has a characteristic inflated look. This is because the protomerite is linked to the epimerite in this area. Approximately 7-8 epicytic folds may be seen per micron in the protomerite area. In the junction of the epimerite and protomerite, there is a short, narrow neck-like structure that remains present (Fig. 2B). Between the intersection of protomerite and deutomerite, there is an obvious constriction. No breakage in epicytic folds has been seen in the septum area. The deutomerite is elongated and continues to be covered with epicytic folds along its length. The density of the epicytic folds is somewhere between 7-9 epicytic folds per micron. The posterior end of the deutomerite is rounded where the epicytic folds end blindly.

Gamont (Figs. 2C-E): The gamonts have a striated pattern running over the surface of their bodies. The epicytic folds are observed to completely cover both the protomerite and the deutomerite. The folds are aligned in parallel with the longitudinal axis of the gamont and are undulating in appearance, comparable to those of trophozoites. The deutomerite exhibits a number of deep longitudinal grooves that run at regular intervals from the septum to the posterior end (Figs. 2C and E). The density of folds is about 7-9 epicytic folds per micron (Fig. 2F).

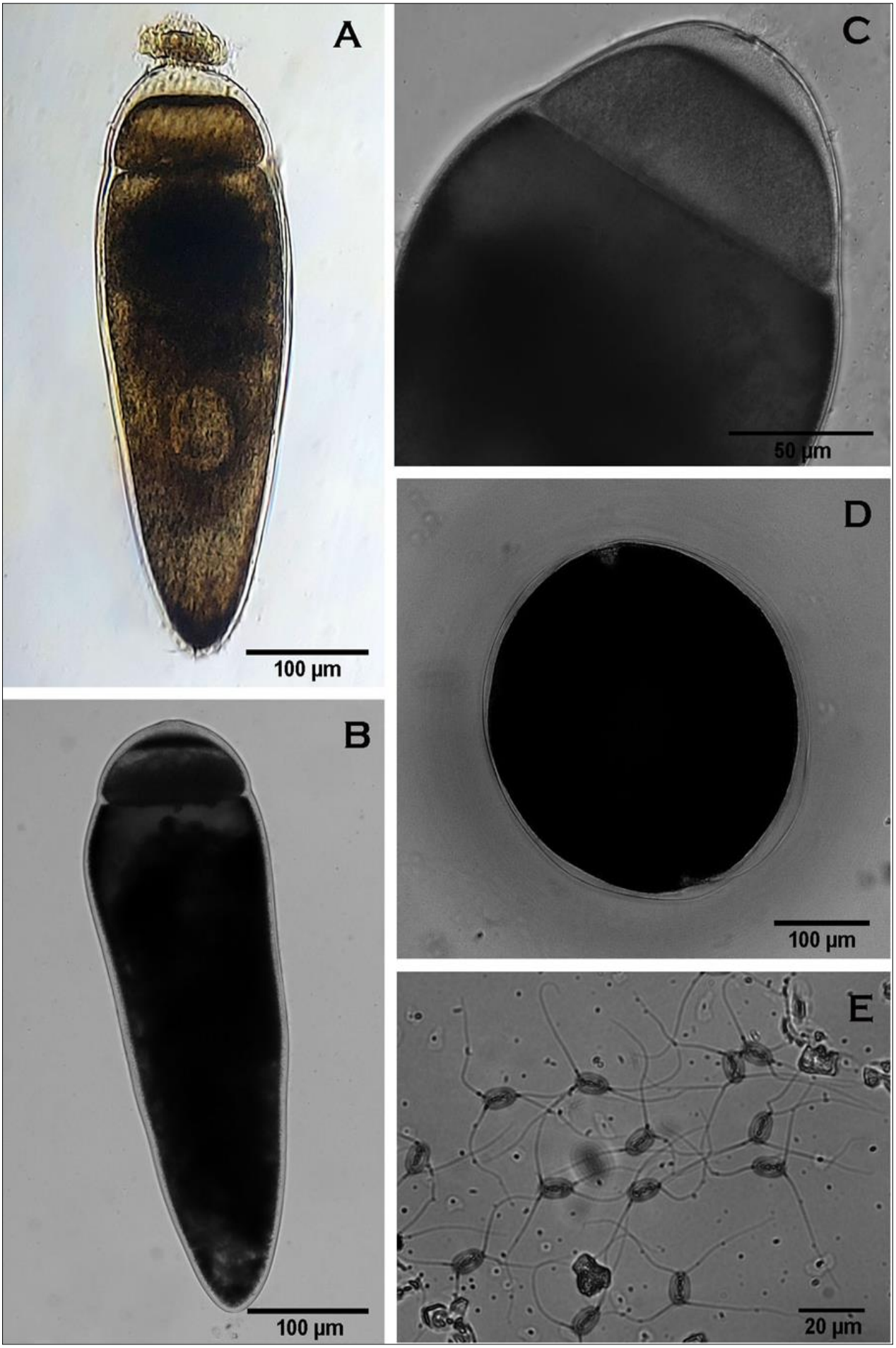

Fig 1: Light micrographs of *Q. indoaiolopii* Haldar and Chakraborty, 1976. A. Mature trophozoite. B. Mature gamont. C. Anterior part of a mature gamont. D. Freshly collected gametocyst. E. Oocysts

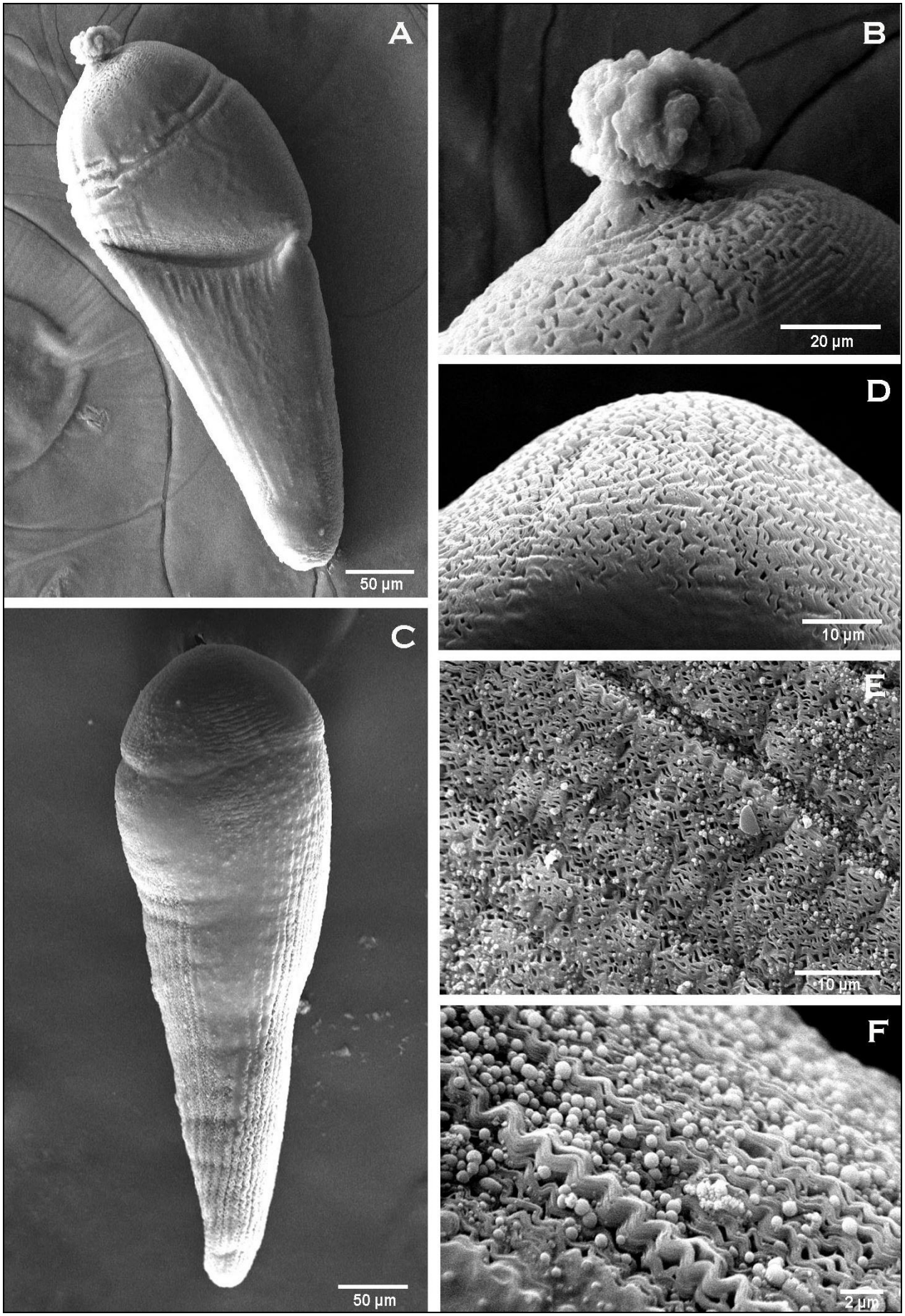

Fig 2: Scanning electron micrographs of *Q. indoaiolopii.* A. Mature trophozoite. B. Epimerite of a mature trophozoite C. Mature gamont. D. Anterior part of a mature gamont. E-F. Scanning electron micrograph with a higher magnification showing the longitudinal epicytic folds, structured in waves.

There was a noticeable shift in the prevalence of *Q. indoaiolopii* during the five seasons (Fig. 3). During the monsoon season, the total infection rate was higher (July to September). It was discovered that eighteen out of forty grasshoppers were found to be infected, having a prevalence of 45%. Infections were also higher in the first few weeks of winter (October to November), eight out of twenty grasshoppers were infected, having a prevalence of 35%. During the summer (May to June), the number of infections was low; two out of twenty individuals were found to be infected (prevalence 10%), and no infections were identified during the early summer (March to April). During the winter (December to February), the prevalence of infections was 4%.

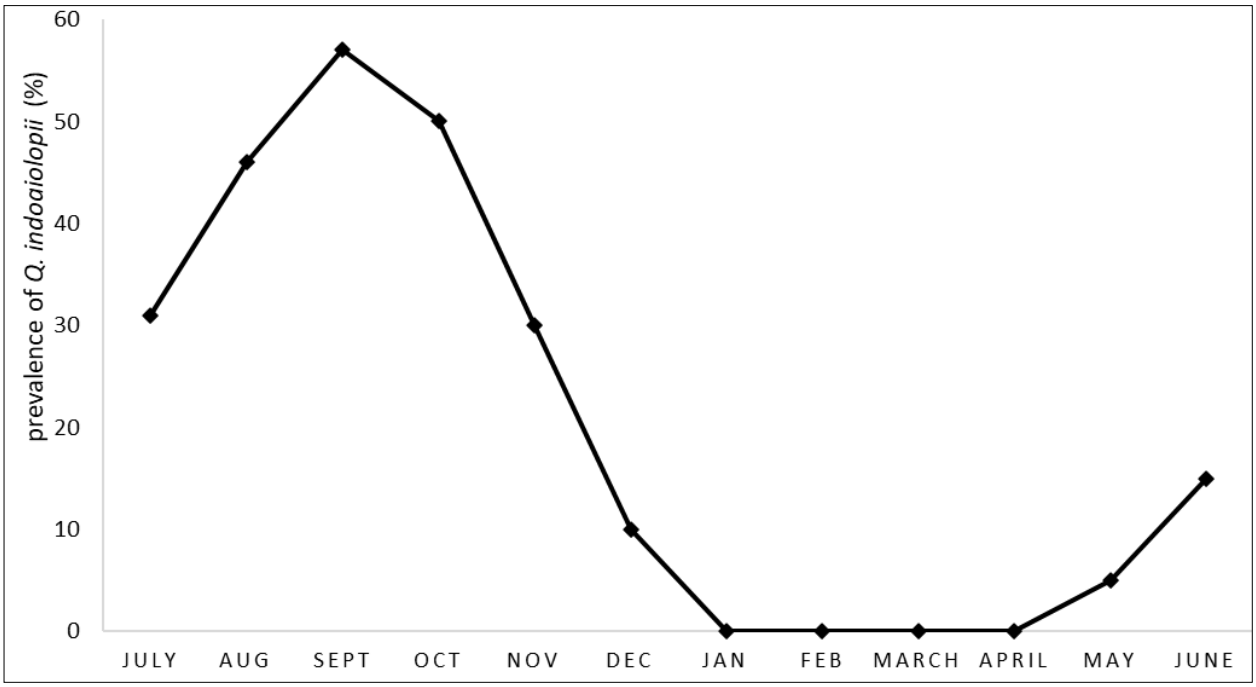

Fig 3: Prevalence of *Q. indoaiolopii* in *A. thalassinus tamulus*.

Discussion

There are now sixteen species that have been identified as belonging to the genus *Quadruspinospora*, and out of those sixteen, fifteen have been documented in India, but there is no available scanning electron microscopic description of any *Quadruspinospora* species reported from India. This study describes the surface ultrastructure of *Q. indoaiolopii*, the first *Quadruspinospora* species from India. Under a light microscope, the pellicle appears to be thick, flexible, and more or less smooth. However, under a scanning electron microscope, the pellicle is seen to have ridges that run the entire length of the body, and at higher magnification, the arrangement of longitudinal epicytic folds is prominent. These epicytic folds have a purpose in the mobility of the cell (Poulpanich and Withyachumnarnkul, 2009) [11]. During gliding, several investigations have shown that longitudinal folds display lateral undulations. (Heller & Weise, 1973; Hildebrand and Vinckier, 1975) [5, 6]. It was hypothesised by Vávra and Small (1969) [16] that gregarines with folds that were more or less straight were less mobile than those with folds that were undulatory. The epicytic folds of *Q. indoaiolopii*, in both the trophozoite and the gamont, appear to be undulating. Similar to *Q. mexicana*, in which the whole trophozoite had longitudinal epicytic folds distributed in waves along the longitudinal axis (Medina-Durán *et al*., 2020) [8], both the trophozoite and gamont of *Q. indoaiolopii* appear to have undulating epicytic folds. The density of folds in *Q. mexicana* was five folds per micron, whereas in *Q. indoaiolopii* it is around seven to nine epicytic folds per micron.

Conclusion

A debate has been ongoing since the beginning about whether or not Quadruspinospora should even be classified as a separate genus (Sarkar 1987, Clopton 2002) [13, 2]; however, Modak *et al*. (2008) [9] established the validity of the genus *Quadruspinospora*. Some disagreements about the correctness of the genus and the distinctions between the species can be settled to some extent by using scanning electron microscopy.

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