

Three New Species of Gregarines (Apicomplexa: Sporozoea: Porosporidae) in the Estuarine Crabs from Kerala, India

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Summary. Different development stages of 3 new species of cephaline gregarines, *Nematopsis messor*, *N. quadratum* and *N. annulipes* infecting the crabs, *Metapograpsus messor* (Forskäl), *Sesarma quadratum* (Fabricius) and *Uca annulipes* Edwards respectively are described and their systematic position discussed.

Key words: crabs, gregarines, *Metapograpsus messor*, *Nematopsis annulipes* sp. n., *N. quadratum* sp. n., *N. messor* sp. n., *Sesarma quadratum*, *Uca annulipes*.

Abbreviations: DW - deutomerite width, LA - length of association, PL - protomerite length, PW - protomerite width, TL - total length.

INTRODUCTION

All cephaline gregarines known to inhabit crabs are included in only 4 genera: *Nematopsis* Schneider, 1892; *Cephaloidophora* Mavrodiadi, 1908; *Pachyporospora* Théodoridès, 1961 and *Stephanospora*, Prema and Janardanan, 1989. Extensive survey of estuarine crabs in Kerala revealed the presence of 3 species of cephaline gregarines belonging to the genus *Nematopsis*. Detailed studies proved that they represent new species. The present paper provides their description.

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MATERIALS AND METHODS

The crabs, *Metapograpsus messor* (Forskäl), *Sesarma quadratum* (Fabricius) and *Uca annulipes* Edwards, collected from sandy and rocky shores of estuaries and river banks in Kerala, were examined for the presence of gregarine parasites. The carapace of each crab was removed, the alimentary tract was then transferred onto a slide and examined for gregarines. Trophozoites and sporadins were recovered from the midgut and gametocytes from the hindgut or from fecal matter. Gametocysts recovered at various stages of development were maintained in moist chamber for further development. The cysts completed development and extruded spores by simple rupture of the cyst wall in 24 h.

Structural details of development stages were studied following the method reported by Prema and Janardanan (1989). Illustrations were made with the aid of a camera lucida; descriptions and measurements are based on a minimum of 20 live specimens. All measurements are in micrometers (μm); mean values are underlined.

Types of all parasites (stained specimens on slides) and their host crabs are deposited in the parasite collections, Parasitology Laboratory, Department of Zoology, University of Calicut, Kerala (India).

RESULTS

Nematopsis messor sp. n. (Fig. 1, Table 1)

Description

Sporadins (Fig. 1.1): biassociative; association caudofrontal; colour milky-white. Syzygy early, linear.

Primites: protomerite shape hemispherical, wider than long, maximum width at posterior end; transparent lens-shaped structure present at anterior end; epicyte uniformly thick, striated; endocyte granular. Septum circular, convex toward deutomerite; constriction at septum inconspicuous. Deutomerite ovoid, narrow behind septum, gradually widens caudally, posterior end broadly rounded; maximum width in posterior half; epicyte uniformly thick; endocyte granular. Nucleus spherical, faintly visible in fresh sporadins, variable in position; endosome single, spherical.

Satellites: lengths on average slightly shorter than primites. Protomerite shape hemispherical, wider than long; epicyte uniformly thick, striated; endocyte granular with transparent lens-shaped structure at anterior end. Septum similar to primate (see above). Deutomerite ovoid, narrow behind septum, gradually widens caudally, posterior end broadly rounded; maximum width at posterior half. Nucleus and endosome similar to primate (see above).

Gametocysts (Fig. 1.2): shape spherical; colour milky-white. Cyst wall double; epicyst thick, hyaline, width uneven, 6.6-11.5; endocyst thin, width uniformly even, 1.6.

Gymnospores (Fig. 1.3): shape spherical; diameter 4.5-5.3; uninucleated bodies arranged radially in rosette pattern around central, hyaline cytoplasm.

Trophozoites (Figs 1.4-1.6): development extracellular. Smallest observed trophozoite (Fig. 1.4) has a hemispherical protomerite and ovoid deutomerite. In an association measuring 129.6 long (Fig. 1.6), the primate measured 70.7 by 37.1 and satellite 58.6 by 37.1.

Taxonomic summary

Type specimens: syntypes, No.Z/Par/G/101; deposited in the parasite collections, Parasitology Laboratory,

Department of Zoology, University of Calicut, Kerala, India.

Type host: *Metapograpsus messor* (Forskäl) (Arthropoda: Crustacea: Grapsidae). Symbiotype: host crab preserved in the Parasitology Laboratory (see address above). Additional hosts: None.

Type locality: India, Kerala, Malppuram district, Kadalundi Estuary, sandy and rocky intertidal. Additional localities in India: Kannur district, Mavilayi and Muzhappilangad; Kozhikode district, Iringal and Kizharyur (all sandy and rocky river banks).

Collection dates: 1988 (February, March, November, December); 1989 (October through December); 1990 (January through April).

Site of infection: intestine.

Prevalence: in 8 of 22 crab hosts examined.

Etymology: named after the species of crab host.

Remarks

Nematopsis messor sp. n. resembles most to *N. raoudi* Vivarès, 1971 from the crab *Portunus latipes*. However, it differs in several significant ways, including the shape of primites and satellites; and in the presence of (1) a prominent lens-shaped structure at the anterior part of the protomerite, (2) a spherical nucleus and (3) a protomerite in the satellites. In addition, the species described above is from a different host and a different geographical locality. This is the first report of gregarines from *Metapograpsus messor* (Forskäl).

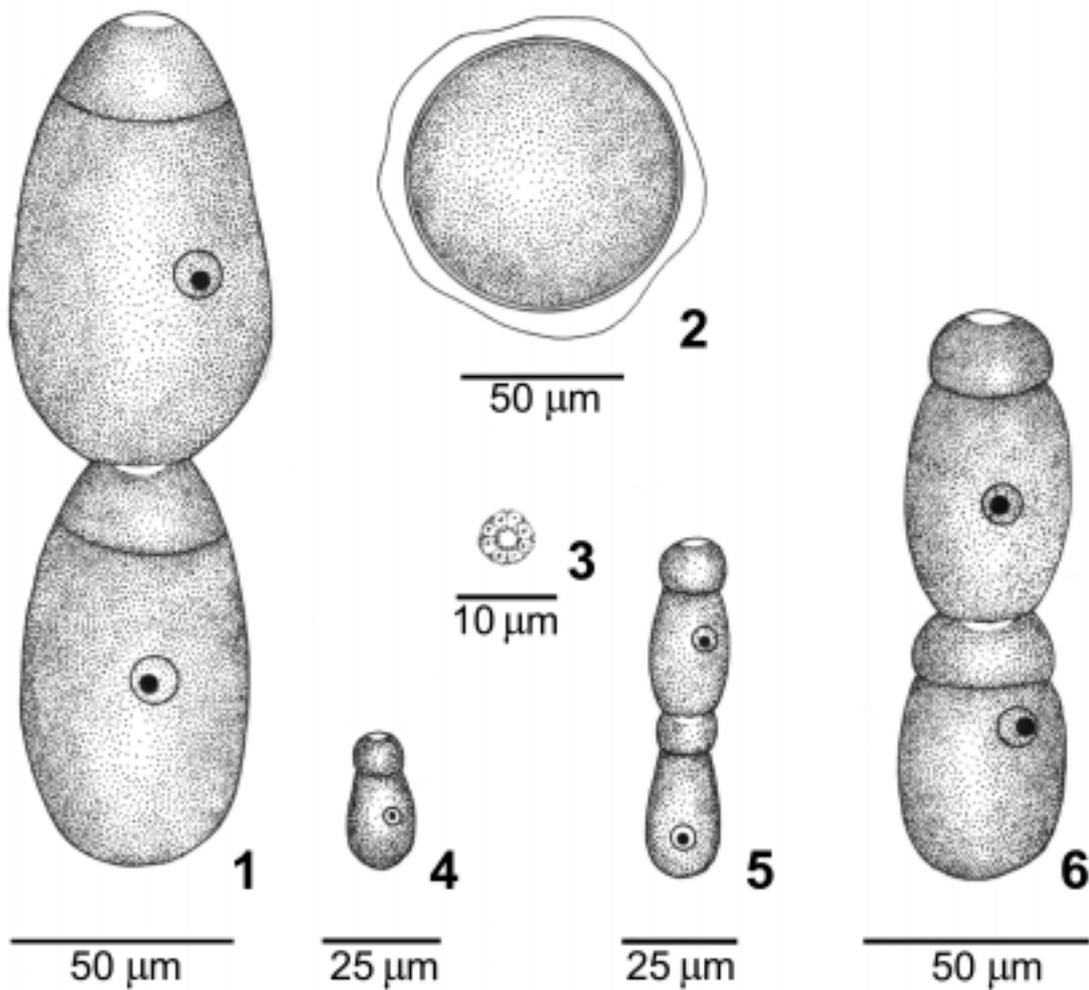
Nematopsis quadratum sp. n. (Fig. 2, Table 1)

Description

Sporadins (Fig. 2.1): biassociative, association caudo-frontal; colour milky-white. Syzygy early, linear.

Primites: always larger than satellites. Protomerite shape hemispherical with a plano-convex to biconvex lens-shaped structure at anterior part; epicyte uniformly thick, striated; endocyte granular. Septum circular, convex toward deutomerite. Deutomerite elongate ovoid, narrow behind septum gradually widens caudally, posterior end broadly rounded; maximum width in posterior half; epicyte hyaline; endocyte granular; a transparent lens-shaped structure at posterior part. Interlocking mechanism between primate and satellite well developed. Nucleus spherical to ovoid.

Satellites: protomerite and septum similar to that of primate (see above). Deutomerite widest behind septum, gradually tapers caudally, posterior end rounded. Nucleus



Figs 1.1-1.6. *Nematopsis messor* sp. n.; 1.1 - sporadins; 1.2 - gametocyst; 1.3 - gymnospore; 1.4 - early trophozoites; 1.5, 1.6 - early associations. Scale bars - 1.1, 1.2, 1.6 - 50 μm; 1.4, 1.5 - 25 μm ; 1.3 - 10 μm

spherical to ovoid, faintly visible in fresh sporadins, variable in position; endosome single, round to ovoid.

Gametocysts (Fig. 2.2): shape spherical; colour milky-white, opaque. Cyst wall double; epicyst thick, hyaline, width uneven, 20.4 - 40.

Gymnospores (Fig. 2.3): shape spherical; diameter 7.5; uninucleated bodies arranged radially in rosette pattern around central, hyaline cytoplasm.

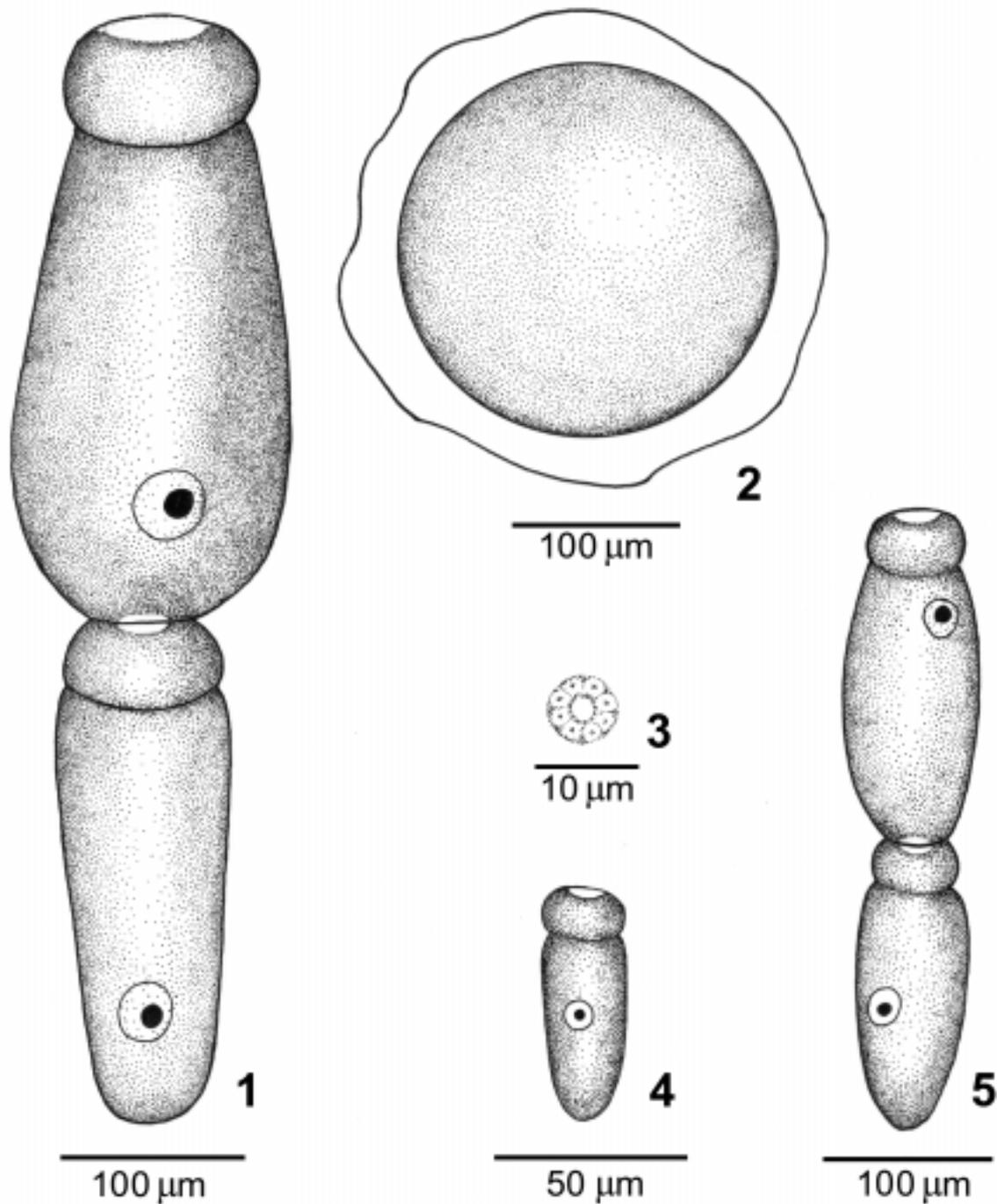
Trophozoites: development extracellular. Smallest observed trophozoite (Fig. 2.4) has a hemispherical, protomerite and cylindrical deutomerite. Largest observed trophozoite was elongate, almost cylindrical, with hemispherical protomerite and elongated deutomerite.

Taxonomic summary

Type specimens: syntypes; No.Z/Par/G/102; deposited in the parasite collections, Parasitology Laboratory, Department of Zoology, University of Calicut, Kerala, India.

Type host: *Sesarma quadratum* (Fabricius) (Arthropoda: Crustacea: Grapsidae). Symbiotype: host crab preserved in the Parasitology Laboratory (see address above). Additional host: none.

Type locality: India, Kerala, Malappuram district, Olippuram rocky river banks. Additional localities in India: Kannur district, Mavilayi and Muzhappilangad; Kozhikode district, Feroke and Ramanattukara;



Figs 2.1-2.5. *Nematopsis quadratum* sp. n.; 1.1 - sporadins; 1.2 - gametocyst; 1.3 - gymnospore; 1.4 - early trophozoites; 1.5 - early association. Scale bars - 1.1, 1.2, 1.5 - 100 μm; 1.4 - 50 μm; 1.3 - 10 μm

Malappuram district, Vazhakkad (all rocky and sandy river banks) and Ernakulam district, Vallarpadam sandy and rocky intertidal.

Collection dates: 1988 (February - March); 1989 (October through December); 1991 (January through April).

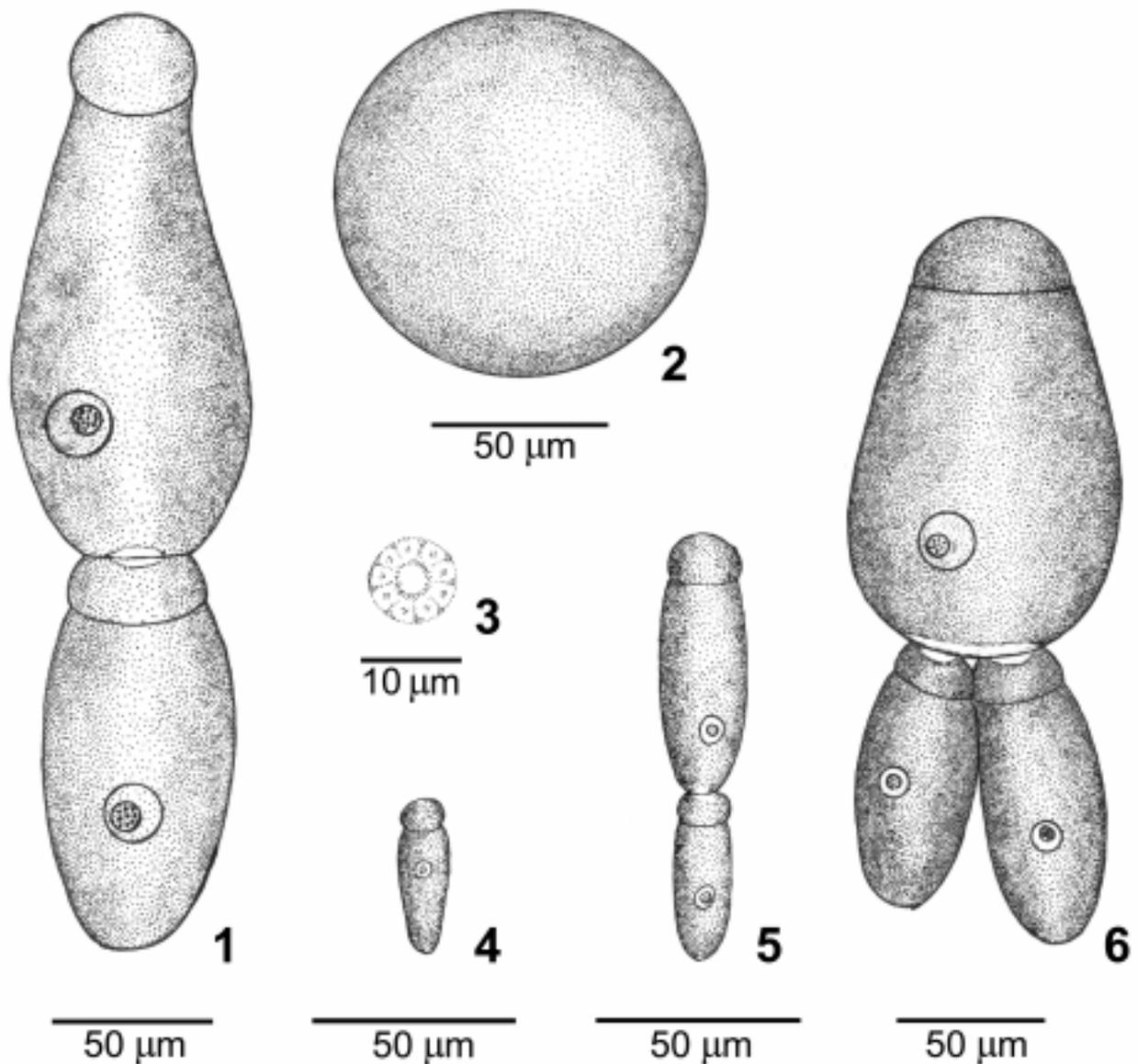
Site of infection: intestine.

Prevalence: in 6 of 31 crab hosts examined.

Etymology: named after the species of crab host.

Remarks

Nematopsis quadratum sp. n. does not resemble any known species of *Nematopsis*. In comparison with



Figs 3.1-3.6. *Nematopsis annulipes* sp. n.; 1.1 - sporadins; 1.2 - gametocyst; 1.3 - gymnospor; 1.4 - early trophozoites; 1.5 - early association; 1.6 - association of three sporadins. Scale bars - 1.1, 1.2, 1.4-1.6 - 50 µm; 1.3 - 10 µm

Nematopsis messor sp. n. (see above) it differs in having (1) larger sporadins, trophozoites, gametocysts and gymnospores, (2) a lens-shaped granule-free structure at the posterior part of primite deutomerite and (3) spherical to ovoid nucleus. The present species is recovered from a different host and this forms the first report of a cephaline gregarine from *Sesarma quadratum* (Fabricius).

***Nematopsis annulipes* sp. n. (Fig. 3, Table 1)**

Description

Sporadins (Fig. 3.1): biassociative, association caudo-frontal; colour milky-white. Syzygy early, linear; occasionally two satellites in syzygy with a primite.

Primites: protomerite shape subspherical to ovoid; epicyte uniformly thick, striated; endocyte granular. Sep-

Table 1. Measurements and ratios of three new species of gregarines of the genus *Nematopsis* found in estuarine crabs in Kerala, India. All measurements taken from fresh specimens; means are underlined

Measurements	<i>N. messor</i>	<i>N. quadratum</i>	<i>N. annulipes</i>
Association Length	114- <u>140.9</u> -178	404- <u>492.7</u> -678	213- <u>259.9</u> -317
Primites:			
Total length	59- <u>72.7</u> -94	177- <u>285.3</u> -370	86- <u>138.4</u> -185
Deutomerite width	30- <u>40.3</u> -58	89- <u>128.1</u> -162	38- <u>59</u> -94
Protomerite length	13- <u>18.6</u> -23	50- <u>59.7</u> -69	17- <u>28.5</u> -36
Protomerite width	23- <u>34</u> -43	54- <u>90.8</u> -121	33- <u>39.9</u> -56
Ratio: PL:TL	1 : 3.9	1 : 4.8	1 : 4.9
Ratio: PW : DW	1 : 1.2	1 : 1.4	1 : 1.5
Satellites:			
Total length	51- <u>68</u> -86	152- <u>207.3</u> -308	101- <u>121.4</u> -147
Deutomerite width	25- <u>33.9</u> -46	50- <u>83.1</u> -117	26- <u>49.2</u> -79
Protomerite length	13- <u>16</u> -20	30- <u>41</u> -56	17- <u>22.1</u> -30
Protomerite width	18- <u>29.9</u> -40	50- <u>74.1</u> -102	25- <u>36.3</u> -43
Ratio: PL : TL	1 : 4.3	1 : 5.1	1 : 5.5
Ratio: PW : DW	1 : 1.1	1 : 1.1	1 : 1.3
Gametocyst (Diameter)	54- <u>66.7</u> -86	139- <u>206.4</u> -246	112- <u>115.5</u> -119
Gymnospor (Diameter)	4.5-5.3	7.5	9
Trophozoites:			
Smallest observed:			
Length:	31.3	56.1	37.1
Protomerite length	9.9	13.2	7.4
Deutomerite length	21.4	42.9	29.7
Largest observed:			
Length:	103.9	300	140
Protomerite length	28	62	28
Deutomerite length	75.9	238	112
Smallest observed			
Association Length:	75.9	385	107
Primate length	39.6	200	66
Satellite length	36.3	185	41

tum circular, convex toward deutomerite. Deutomerite narrow behind septum gradually dilates caudally, posterior end broadly round or flat; epicyte longitudinally striated; endocyte granular with a transparent lens-shaped structure at posterior end. Nucleus spherical, variable in position; endosome single, spherical, contains 8-12 granules.

Satellites: protomerite shape rectangular, broader than long; anterior margin flat; deutomerite shape elongate ovoid, which is widest at the middle; epicyte uniformly thick; endocyte granular. Nucleus and endosome similar to primate (see above).

Association of 3 sporadins (Fig. 3.6): two satellites in syzygy with a primate. Primate ovoid, measured 151.8; one satellite 87.5 and the other satellite 102.3 in length.

Gametocysts (Fig. 3.2): shape spherical to ovoid; colour milky-white; cyst wall single, hyaline.

Gymnospres (Fig. 3.3): shape spherical, uninucleated bodies arranged radially in a rosette pattern around a central, hyaline cytoplasm.

Trophozoites: development extracellular. Smallest observed trophozoite (Fig. 3.4) narrow, elongate ovoid.

Taxonomic summary

Type specimens: syntypes; No. Z/Par/G/103; deposited in the parasite collection, Parasitology Laboratory, Department of Zoology, University of Calicut, Kerala, India.

Type host: *Uca annulipes* Edwards (Arthropoda: Crustacea: Ocypodidae). Symbiotype: host crabs preserved in the Parasitology Laboratory (see address above). Additional host: none.

Type locality: India, Kerala, Malappuram district, Olippuram River, muddy and sandy shores. Additional localities in India: Kannur District, Mavilayi and

Muzhappilangad; Kozhikode district, Chaliyam and Malappuram district, Ramanattukara Kadalundi and Vazhakkad (all sandy and muddy river banks).

Collection dates: 1988 (February-March); 1989 (October through December); 1991 (January through April).

Site of infection: intestine

Prevalence: in 9 of 44 crab hosts examined.

Etymology: named after the species of crab host.

Remarks

Nematopsis annulipes recovered from *Uca annulipes* Edwards, also does not resemble any known species in the genus that have been described from crabs. In some characters it superficially resembles *Nematopsis quadratum* sp. n. (see above). From a comparative study of characters, it is evident that unlike *N. quadratum* the present form has (1) subspherical to ovoid primite protomerite without lens-shaped structure, (2) elongate ovoid satellite deutomerite which is widest at the middle, (3) spherical nuclei, (4) spherical to ovoid and single walled gametocysts and (5) larger gymnospires.

DISCUSSION

Cephaline gregarines infecting crabs are grouped into two families, namely the Porosporidae Labbé, 1899 and Cephaloidophoridae Kamm, 1922. The gregarines recovered from estuarine crabs in the present study have extracellular development and their gametocysts formed gymnospires. These characters warrant placing the gregarines in the family Porosporidae. Members of the Porosporidae are heteroxenous and molluscs are the only known intermediate hosts. Sporogonic development in the intermediate hosts of many species remains

unknown. In cases where sporogony is unknown generic placement is based on the morphology of vegetative stages in the digestive tract of crustacean hosts. Molluscan hosts of the present gregarines could not be discovered even after repeated attempts. Hence generic designation was based on the morphology of the trophozoites, sporadins and gymnospires. The family Porosporidae includes four genera, *Porospora*, *Nematopsis*, *Pachyporospora* and *Stephanospora*. Amongst these the genus *Porospora* has not been reported from crabs. Trophozoites and sporadins of the present gregarines do not resemble either *Pachyporospora* or *Stephanospora* but bear the requisite characters of *Nematopsis* and are, therefore, assigned to this genus.

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