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

Puthampurayil K. PRASADAN¹ and Kuniyil P. JANARDANAN²

¹Department of Zoology, Mary Matha Arts & Science College, Vemom (P.O.), Mananthavady; ²Parasitology Laboratory, Department of Zoology, University of Calicut, Kerala, India

Summary. Different development stages of 3 new species of cephaline gregarines, Nematopsis messor, N. quadratum and N. annulipes infecting the crabs, Metapograpsus messor (Forskal), 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.

Address for correspondence: Puthampurayil K. Prasadan, Department of Zoology, Mary Matha Arts & Science College, Vemom (P.O.), Mananthavady-670645, Kerala, India; E-mail: drprasadan@yahoo.co.in

MATERIALS AND METHODS

The crabs, Metapograpsus messor (Forskal), 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 gametocysts 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 primite (see above). Deutomerite ovoid, narrow behind septum, gradually widens caudally, posterior end broadly rounded; maximum width at posterior half. Nucleus and endosome similar to primite (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 primite 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 (Forskål) (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 Kizhariyur (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 Vivårès, 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 (Forskål).

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

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

Primites: always larger than satellites. Protomerite shape hemispherical, wider than long; epicyte uniformly thick, striated; endocyte granular with transparent lens-shaped structure at anterior end. Septum similar to primite (see above). Deutomerite ovoid, narrow behind septum, gradually widens caudally, posterior end broadly rounded; maximum width at posterior half. Nucleus and endosome similar to primite (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 primite 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 (Forskål) (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 Kizhariyur (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 Vivårès, 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 (Forskål).
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;
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...
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 primites 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 caudofrontal; colour milky-white. Syzygy early, linear; occasionally two satellites in syzygy with a primites.
Primites: protomerite shape subspherical to ovoid; epicyte uniformly thick, striated; endocyte granular. Sep-
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 primite (see above).

Association of 3 sporadins (Fig. 3.6): two satellites in syzygy with a primite. Primites 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.

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

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**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

<table>
<thead>
<tr>
<th>Measurements</th>
<th>N. messor</th>
<th>N. quadratum</th>
<th>N. annulipes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Association</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>114-140.9-178</td>
<td>404-492.7-678</td>
<td>213-259.9-317</td>
</tr>
<tr>
<td><strong>Primites:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total length</td>
<td>59-72.7-94</td>
<td>177-285.3-370</td>
<td>86-138.4-185</td>
</tr>
<tr>
<td>Deutomerite width</td>
<td>30-40.3-58</td>
<td>89-128.1-162</td>
<td>38-59-94</td>
</tr>
<tr>
<td>Protomerite length</td>
<td>13-18.6-23</td>
<td>50-59.7-69</td>
<td>17-28.5-36</td>
</tr>
<tr>
<td>Protomerite width</td>
<td>23-34-43</td>
<td>54-90-8-121</td>
<td>33-39.9-56</td>
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<tr>
<td>Ratio: PL : TL</td>
<td>1 : 3.9</td>
<td>1 : 4.8</td>
<td>1 : 4.9</td>
</tr>
<tr>
<td>Ratio: PW : DW</td>
<td>1 : 1.2</td>
<td>1 : 1.4</td>
<td>1 : 1.5</td>
</tr>
<tr>
<td><strong>Satellites:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total length</td>
<td>51-68-86</td>
<td>152-207.3-308</td>
<td>101-121.4-147</td>
</tr>
<tr>
<td>Deutomerite width</td>
<td>25-33.9-46</td>
<td>50-83.1-117</td>
<td>26-49.2-79</td>
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<td>Protomerite length</td>
<td>13-16-20</td>
<td>30-41-56</td>
<td>17-22.1-30</td>
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<tr>
<td>Protomerite width</td>
<td>18-29.9-40</td>
<td>50-74.1-102</td>
<td>25-36.3-43</td>
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<tr>
<td>Ratio: PL : TL</td>
<td>1 : 4.3</td>
<td>1 : 5.1</td>
<td>1 : 5.5</td>
</tr>
<tr>
<td>Ratio: PW : DW</td>
<td>1 : 1.1</td>
<td>1 : 1.1</td>
<td>1 : 1.3</td>
</tr>
<tr>
<td>Gametocyst (Diameter)</td>
<td>54-66.7-86</td>
<td>139-206.4-246</td>
<td>112-115.5-119</td>
</tr>
<tr>
<td>Gymnospore (Diameter)</td>
<td>4.5-5.3</td>
<td>7.5</td>
<td>9</td>
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<tr>
<td><strong>Trophozoites:</strong></td>
<td></td>
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<tr>
<td>Smallest observed:</td>
<td></td>
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<tr>
<td>Length:</td>
<td>31.3</td>
<td>56.1</td>
<td>37.1</td>
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<tr>
<td>Protomerite length</td>
<td>9.9</td>
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<td>7.4</td>
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<td>Deutomerite length</td>
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<td>29.7</td>
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<td>Largest observed:</td>
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<tr>
<td>Length:</td>
<td>103.9</td>
<td>300</td>
<td>140</td>
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<tr>
<td>Protomerite length</td>
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<td>28</td>
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<tr>
<td>Deutomerite length</td>
<td>75.9</td>
<td>238</td>
<td>112</td>
</tr>
<tr>
<td>Smallest observed:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Association Length:</td>
<td>75.9</td>
<td>385</td>
<td>107</td>
</tr>
<tr>
<td>Primate length</td>
<td>39.6</td>
<td>200</td>
<td>66</td>
</tr>
<tr>
<td>Satellite length</td>
<td>36.3</td>
<td>185</td>
<td>41</td>
</tr>
</tbody>
</table>
Gregarine new species

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 primate 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 gymnospores.

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 gymnospores. 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 gymnospores. 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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