

## ***Myxoproteus cheni* sp. n. and *Sinuolinea mai* sp. n. (Myxosporea: Sinuolineidae) Parasitic in the Urinary Bladder of Marine Fish (*Thamnaconus septentrionalis* Gunther, 1877) from the Yellow Sea, off the Qingdao Coast of China**

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**Summary.** Two new species of myxosporeans (Myxosporea: Sinuolineidae), *Myxoproteus cheni* sp. n. and *Sinuolinea mai* sp. n., parasitized in marine fishes collected from the Yellow Sea, off the Qingdao coast of China, are described. Both species, including spores and plasmodia were found in the urinary bladder of the host, *Thamnaconus septentrionalis* Gunther, 1877. The diagnostic features of *Myxoproteus cheni* sp. n.: trophozoites monosporous or disporous; spore inversely pyramidal or triangular to subspherical with smooth surface, fine sutural line straight or slightly sinuous,  $14.0 \pm 0.8$  (12.8-15.3) x  $12.8 \pm 1.3$  (11.2-15.0) x  $12.5 \pm 1.5$  (11.2-15.0)  $\mu\text{m}$  in size; two spherical polar capsules located anteriorly and conspicuously separated from each other,  $3.6 \pm 0.4$  (3.2 - 4.2)  $\mu\text{m}$  in diameter; coelozic. *Sinuolinea mai* sp. n.: trophozoites disporous; spore body inversely pyramidal to spherical with smooth surface, sutural line highly sinuous,  $22.4 \pm 0.5$  (21.7-23.0) x  $19.5 \pm 0.5$  (18.8-20.2)  $\mu\text{m}$  in size; two spherical polar capsules located anteriorly and conspicuously separated from each other,  $5.8 \pm 0.4$  (5.0-6.3)  $\mu\text{m}$  in diameter; coelozic.

**Key words:** marine fishes, *Myxoproteus cheni* sp. n., Myxozoa, *Sinuolinea mai* sp. n., Sinuolineidae, *Thamnaconus septentrionalis*, urinary bladder.

### INTRODUCTION

Protozoan parasites from the Yellow Sea have never been paid attention until recently, although enormous development of commercial fish farming has been taking place in this region in last decades (Meng Qingxian 1996, Zhao and Song 1999, Zhao *et al.* 2000).

In view of the recognized pathogenicity inflicted by many protozoan fish parasites on commercial and aquarium fish, it is desirable to obtain data on protozoa infecting fish in this region.

During the recent investigations on the fauna of protozoan parasites from marine fishes off the coast of the Yellow Sea, some myxosporean parasites were found. Among those, compared with their known congeners, some organisms obtained are believed to be new members of the family Sinuolineidae. This communication brings description of two new myxosporean species of the genera *Sinuolinea* and *Myxoproteus* from a

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marine fish, *Thamnaconus septentrionalis* Gunther, 1877.

## MATERIALS AND METHODS

The host fish *Thamnaconus septentrionalis* Gunther, 1877 was obtained on two occasions in March to July, 1998 and April to June, 1999 in coastal water of the Yellow Sea off the Qingdao (Tsingtao), China. All body organs were examined in fresh mounts, and myxosporeans were observed and measured in fresh state. Preparations of parasites were studied from air-dried smears stained with Giemsa (1:20) after fixation in absolute methanol. The mixture of glycerin - ethyl alcohol 70% - formalin (G. : A. : F. = 12 : 108 : 5) was used to show the morphological structure of mature spores and immature spores as well as the plasmodia. All specimens were observed and measured at magnification of  $\times 1250$ . Illustrations were drawn with the aid of camera Lucida and computer program Photoshop 5.0.

## RESULTS AND DISCUSSION

### *Myxoproteus cheni* sp. n. (Figs. 1-7)

Diagnosis: Trophozoites monosporous or disporous; spore inversely pyramidal or triangular to subspherical with smooth surface, sutural line fine straight or slightly sinuous,  $14.0 \pm 0.8$  (12.8-15.3)  $\times$   $12.8 \pm 1.3$  (11.2-15.0)  $\times$   $12.5 \pm 1.5$  (11.2-15.0)  $\mu\text{m}$  in size; two spherical polar capsules located anteriorly and conspicuously separated from each other,  $3.6 \pm 0.4$  (3.2 - 4.2)  $\mu\text{m}$  in diameter; coelozic.

Dedication: this species is named after Professor Chen Qiliu (Chen Chihleu), who kindly instructed the first author and who is Protozoologist and Myxosporologist of Institute of Hydrobiology, Academy of Sciences, to express our respects.

Trophozoites: early stage of sporogonic pseudo-plasmodia small and irregular in shape, with hyaline ectoplasmic layer, about  $16.5 \times 13.5 \mu\text{m}$  in size (Fig. 1); late stage of plasmodia with developing mono- and di-spore, which measure  $25.9-86.7 \times 15.9-56.7 \mu\text{m}$  in size; *in vivo* cells usually possessing two or three filament-shaped pseudopodia at both poles like amoeba (Figs. 1, 7).

Spore: inversely pyramidal or triangular to subspherical with smooth surface and straight or slightly curved sutural line; two spherical polar capsules set well apart; one binucleated sporoplasm in the spore cavity. Neither iodophilous vacuole in sporoplasm nor mucous envelope around spore observed.

Host: *Thamnaconus septentrionalis* Gunther, 1877.  
Site of infection: urinary bladder cavity.

Locality: coastal waters of Qingdao (36° 08' N, 120° 43' E) of the Yellow Sea, China.

Prevalence: one of 10 fish examined was infected (10%).

Ecological features: salinity about 32‰, water temperature 11-12°C.

Date of sampling: 1998. 5. 25, 1999. 5. 23.

Pathogenicity: unknown.

Type-specimens: syntypes on slide No. qd-19980525, deposited to the Laboratory of Protozoology, College of Fisheries, Ocean University of Qingdao, Qingdao, China.

### Taxonomic affinities

Class: Myxosporia Bütschli, 1881

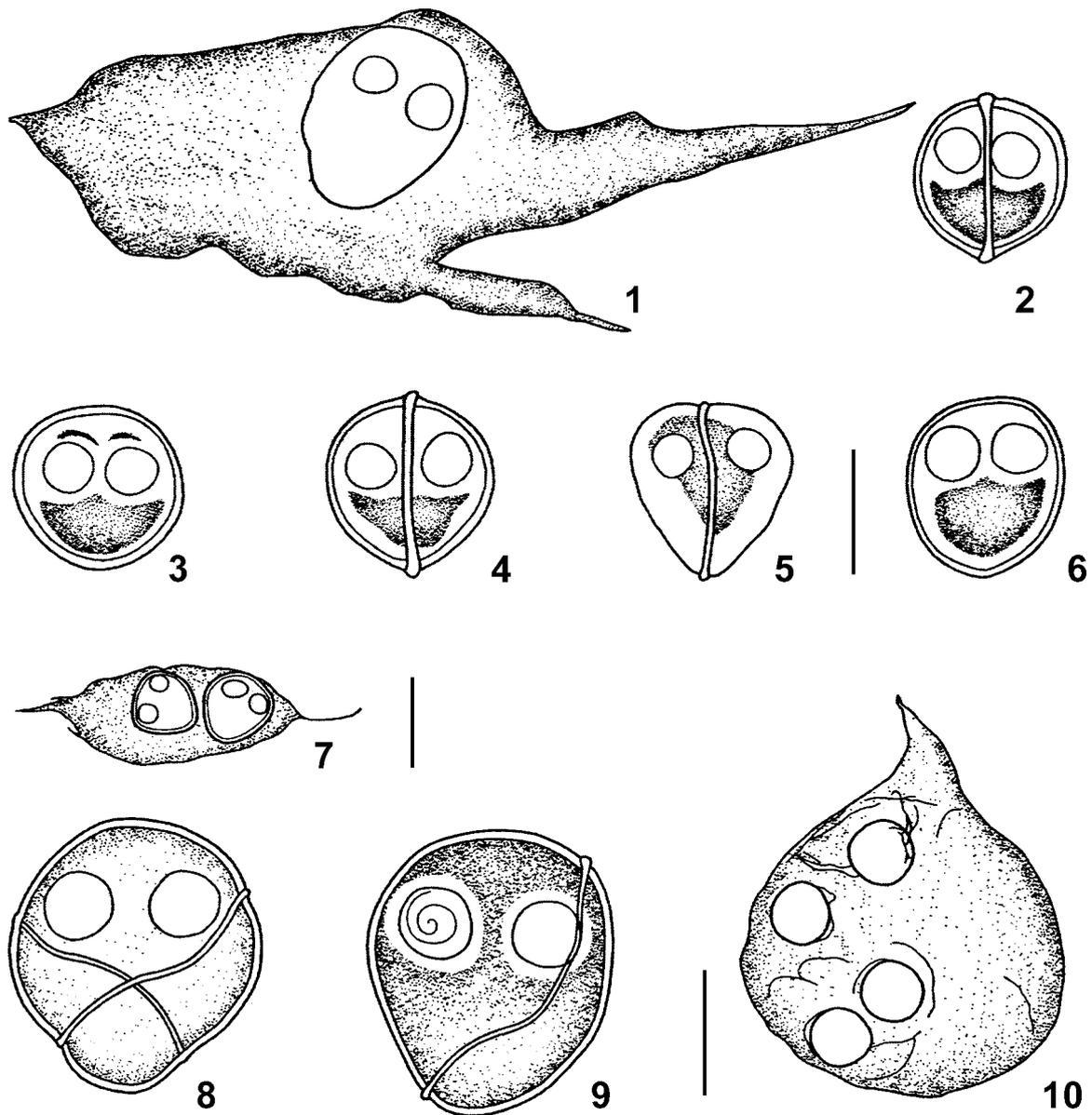
Order: Bivalvulida Schulman, 1959

Family: Sinuolineidae Schulman, 1959

Genus: *Myxoproteus* Doflein, 1898

Doflein (1898) established *Myxoproteus* firstly, then Davis (1917) emended its diagnostic characterization. Up to now, 18 species were described (Thelohan, 1895; Doflein, 1898; Davis, 1917; Fantham *et al.*, 1940; Schulman, 1953, 1966; Kabata, 1962; Yoshino and Noble, 1973; Yoshino and Moser, 1974; Evdokimova, 1977; Moser and Noble, 1977; Sankurathri, 1977; Kovaleva and Gayevskaya, 1979, 1982; Kovaleva, 1989; Wierzbicka, 1986; Sarkar, 1996), and majority of them always live in the urinary bladder and urinary system of marine fishes, except *M. ambiguum* (Thelohan, 1895) Doflein, 1898 and *M. myoxocephali* Fantham *et al.*, 1940 in the gall bladder of marine fishes. Species of this genus share the following characters: spore inversely pyramidal, triangular to spherical in sutural view with rounded outline; anterior end broad and more or less truncated; straight or slightly sinuous sutural line; thick-walled shell with or without projections; two spherical polar capsules well apart; binucleated sporoplasm; trophozoites mono- and disporic (Kudo 1920, Schulman 1966, Lom and Dyková 1992).

In the light of the morphologically similar forms, following species should be compared with our species: *Myxoproteus elongatus* Schulman, 1953, *M. meridionalis* Evdokimova, 1977, and *M. cujaeus* Sarkar, 1996. *M. elongatus* differs from our form in having elongated inversely pyramidal spore, smaller size in width (6.0-7.0) and in thickness (7.0-8.5) of spores as well as relatively smaller diameter of polar capsules (2.0-3.5). *M. meridionalis* appears distinctly different



**Figs. 1-10.** Illustrations of *Myxoproteus cheni* sp. n. and *Sinuolinea mai* sp. n. **1-7-** *Myxoproteus cheni* sp. n. **1-** plasmodium with one developing spore; **2, 4, 5 -** mature spore from lateral view; **3, 6 -** mature spore from oblique view. **7 -** plasmodium with two developing spores. **8-10 -** *Sinuolinea mai* sp. n. **8, 9 -** mature spore from lateral view; **10 -** plasmodium with two early-stage spore. Scale bars - 10  $\mu$ m (**1-6, 8-10**), 20  $\mu$ m (**7**)

from our species in bearing ellipsoidal spores and smaller width of spore (8.0-9.3). *M. cujaeus* can be distinguished by possessing strongly sinuous sutural line and smaller spores (9.0-12.0 x 8.0-10.0). Our new species is distinguished by the following combined features: A) larger dimension of spore (12.8-15.3 x 11.2-15.0);

B) inversely pyramidal or triangular to spherical and C) straight or slightly sinuous sutural line. Furthermore, all these 4 species are from different hosts and regions: *M. elongatus* from *Anarhichas lupus* Linnaeus, 1758 and *Licichthys deuyiculatus* of Barents Sea, White Sea; *M. meridionalis* in *Merluccius hubbsi* Marini,

**Table 1.** Comparison of the related species of *Myxoproteus* Doflein, 1898 with *Myxoproteus cheni* sp. n. L - length; W - width; T - thickness; D - diameter (all in  $\mu\text{m}$ )

Species	Host	Locality	Localization	Shape of spore	Size of spore	Size of PC <sup>a</sup>	Data resource
<i>Myxoproteus cheni</i> sp. n.	<i>Thamnaconus septentrionalis</i>	The Yellow Sea, China	Urinary bladder	Inversely pyramidal or triangular to spherical	L: $14.0 \pm 0.8$ (12.8-15.3) <sup>b</sup> W: $12.8 \pm 1.3$ (11.2-15.0) T: $12.5 \pm 1.5$ (11.2-15.0)	D: $3.6 \pm 0.4$ (3.2 - 4.2)	This study
<i>M. elongatus</i> Schulman, 1953	<i>Anarhichas lupus</i> ; <i>Licichthys deuyiculatus</i>	Barents Sea; White Sea	Urinary bladder	Elongated pyramidal	L: 10.0-13.5 W: 6.0-7.0 T: 7.0-8.5	D: 2.0-3.5	Schulman, 1953
<i>M. meridionalis</i> Evdokimora, 1977	<i>Merluccius hubbsi</i>	Southwest Atlantic	Urinary bladder	Ellipsoidal	L: 10.0-11.0 W: 8.0-9.3	L: 3.3 W: 2.7	Evdokimora, 1977
<i>M. cujaeus</i> Sarkar, 1996	<i>Macropsinosa cuja</i>	West Bengal, India	Urinary bladder	Spherical	L: $10.5 \pm 0.8$ (9.0-12.0) W: $9.2 \pm 0.8$ (8.0-10.0)	D: $3.3 \pm 0.4$ (3.0-4.0)	Sarkar, 1996

<sup>a</sup> polar capsule; <sup>b</sup>Mean  $\pm$  SD (Min-Max)

**Table 2.** *Sinuolinea mai* sp. n. Characteristics of spores found in filefish *Thamnaconus septentrionalis* compared with other *Sinuolinea* spp. L - length; W - width; D - diameter (all in  $\mu\text{m}$ )

Species	Spore size	Polar capsule size	Pseudoplasmodium size	Infection locus	Host	Locality	Data resource
<i>Sinuolinea mai</i> sp. n.	L: $22.4 \pm 0.5$ (21.7-23.0) <sup>a</sup> W: $19.5 \pm 0.5$ (18.8-20.2)	D: $5.8 \pm 0.4$ (5.0 - 6.3)	L: 29.4 W: 25.3	Urinary bladder	<i>Thamnaconus septentrionalis</i>	The Yellow Sea, China	This study
<i>S. dimorpha</i>	D: 15.0	D: 4.5	L: 57.0 W: 90.0	Urinary bladder	<i>Cynoscion regalis</i>	Beaufort region, U.S.A.	Davis, 1917
<i>S. triangularta</i>	D: 14.0-15.0	D: 5.0-6.5	D: 20.0	Urinary bladder	<i>Sphaeroides vermicularis</i>	Sea of Japan	Schulman, 1966
<i>S. magna</i>	D: 19.0-30.0	D: 5.0-7.5	-	Urinary bladder	<i>Coryphaenoides acrolepis</i> ; <i>C. pectoralis</i>	Southern California, U.S.A.	Yoshino and Noble, 1973
<i>S. indica</i>	D: 10.0-13.5	D: 3.0-4.0	L: 20.0-25.0 W: 13.5-17.0	Urinary bladder	<i>Pseudosciaena coibor</i>	Hooghly estuary, West Bengal, India	Sarkar, 1997

<sup>a</sup> Mean  $\pm$  SD (Min-Max)

1932 of southwest Atlantic; *M. cujaeus* Sarkar, 1996 from *Macrospinosa cuja* Ham. in West Bengal, India; *M. cheni* sp. n. from *Thamnaconus septentrionalis* Gunther, 1877 of the Yellow Sea, China (Table 1).

***Sinuolinea mai* sp. n. (Figs. 8-10)**

Diagnosis: trophozoites disporous; spore body inversely pyramidal to spherical with smooth surface, sutural line highly sinuous, thin and slender suture ridge,  $22.4 \pm 0.5$  (21.7-23.0) x  $19.5 \pm 0.5$  (18.8-20.2)  $\mu\text{m}$  in size; two spherical polar capsules located anteriorly and conspicuously separated from each other,  $5.8 \pm 0.4$  (5.0-6.3)  $\mu\text{m}$  in diameter; coelozic.

Dedication: this species is named after Professor Ma Chenglun, who is Parasitologist and Myxosporologist in the Department of Biology, Chongqing Normal College.

Trophozoites: early stage of plasmodium not observed; late stage drop-like or comma-shaped; *in vivo* cells usually possessing one pseudopodium like amoeba (Fig. 10), always with disporous plasmodium, which measures 29.4 x 25.3  $\mu\text{m}$ .

Spores: body of mature spores inversely pyramidal to subspherical in shape from sutural view; sutural line very sinuous and twisted on its axis (Figs. 8, 9); shell valves thin-walled with smooth surface; spore cavity with binucleated mass of sporoplasm which is often finely granulated; two polar capsules spherical or subspherical, about equal size and located anteriorly far away from each other (Figs. 8, 9); neither iodophilous vacuole in sporoplasm nor mucous envelope around spore to spot.

Host: *Thamnaconus septentrionalis* Gunther, 1877

Site of infection: urinary bladder.

Locality: coastal waters off Qingdao (36° 08' N, 120° 43' E) of the Yellow Sea, China.

Prevalence: one of 10 fish examined was infected (10%).

Ecological features: salinity about 32‰, water temperature 11-13°C.

Date of sampling: 1998.6.11; 1999.5.23.

Pathogenicity: not apparent.

Type-specimens: one slide as holotype (No. qd-19980611) is deposited in the Laboratory of Protozoology, College of Fisheries, Ocean University of Qingdao, Qingdao, China.

**Taxonomic affinities**

Class: Myxosporia Bütschli, 1881

Order: Bivalvulida Schulman, 1959

Family: Sinuolineidae Schulman, 1959

Genus: *Sinuolinea* Davis, 1917

Davis (1917) established *Sinuolinea*, in which the spores are spherical or subspherical with 2 nearly spherical polar capsules, and sutural line forms a prominent sinuous ridge around the spore. Since then 15 species were identified (Davis, 1917; Basikalowa, 1932; Tripathi, 1948; Shulman, 1953, 1966; Yoshino and Noble, 1973; Gayevskaya and Kovaleva, 1979; Karatayev and Iskov, 1984, 1985; Ibrahimov, 1988; Moser, Kent and Dennis, 1989; El-Matbouli and Hoffmann, 1994; Sarkar, 1997). All species live in the marine fishes, except *Sinuolinea tetraodoni* El-Matbouli and Hoffmann, 1994.

According to the spore shape, four species, *Sinuolinea dimorpha* Davis, 1917, *Sinuolinea triangularis* Shulman, 1966, *Sinuolinea indica* Sarkar, 1997 and *Sinuolinea magna* Yoshino & Noble, 1973 show similarity with the present species. However, the spores of *S. dimorpha* (Sp. 15.0  $\mu\text{m}$ , Pc. 4.5  $\mu\text{m}$ ), *S. triangularis* (Sp. 14.0-15.0  $\mu\text{m}$ , Pc. 5.0-6.5  $\mu\text{m}$ ), *S. indica* (Sp. 12.0  $\mu\text{m}$ , Pc. 3.0-4.0  $\mu\text{m}$ ) exhibit clear difference in the dimension of spores and the polar capsule from *S. mai* (Sp. 21.7-23.0 x 18.8-20.2  $\mu\text{m}$ , Pc. 5.0-6.3  $\mu\text{m}$ ). With reference to dimension, the spore of *S. magna* shows similarity to that of our form, the former is, however, distinctly different from the latter in the shape of spore (spherical), and sutural ridge (thick and strong). In addition, the 4 species mentioned above are obtained from different hosts and regions (Table 2).

Two new species of *Sinuolineidae myxosporean* described in this paper, are from the same host, but they really differ from each other in shape and size of spore. Especially, they have different sutural line, sutural line strongly sinuous in the spore of *S. mai* and sutural line fine straight or slightly sinuous in the spore of *M. cheni*. In view of such differences, the two myxosporeans species are considered as two new species relatively.

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