# *Myxobolus goensis* n. sp. (Myxozoa, Myxosporea, Myxobolidae), a parasite of the gills of *Mugil cephalus* (Osteichthyes, Mugilidae) from Goa, India

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**Summary:**

Two species of *Myxobolus* are reported from the gills of *Mugil cephalus* collected at Goa, India: *M. goensis* n. sp. and *M. parvus* Shulman, 1962. *Myxobolus goensis* n. sp. forms digitiform or rounded plasmodia between the gill rakers. Their spores are oval in frontal view, with tapered anterior extremity, and lemon-shaped in lateral view, measuring 9.7 (9.5-10.5) µm in length, 6.6 (6-7.5) µm in width, and 5.2 (5-6) µm in thickness. The polar capsules are pyriform and unequal in size. The larger ones are 5.3 (4.5-6) µm long and 2.4 (2-3) µm wide; the smaller ones are 2.4 (2-3) µm long and 1.8 (1.5-2) µm wide. The polar filament forms five turns aligned perpendicularly to the longitudinal axis of the larger polar capsules. Within the smaller polar capsules the polar filament is difficult to observe and, apparently, forms three coils. The spores are distinctly different from other *Myxobolus* species infecting *M. cephalus* and other *Mugil* spp. Furthermore, the present material is also different from 204 *Myxobolus* species presenting differently sized polar capsules, representing nearly all the known species with this characteristic. The fact that only the *M. cephalus* specimens were infected among a sample of 206 fish specimens, comprising 27 different species, strongly suggests that this parasite is specific to *M. cephalus*.

**KEY WORDS :** Myxosporea, *Myxobolus goensis* n. sp., *Mugil cephalus*, fish, Goa, India.

***Résumé :*** *Myxobolus goensis* n. sp. (Myxozoa, Myxosporea, Myxobolidae), parasite des branchies de *Mugil cephalus* (Osteichthyes, Mugilidae) à Goa, en Inde

*Deux espèces de* Myxobolus *sont observées au niveau des branchies de* Mugil cephalus *collecté à Goa, en Inde :* M. goensis *n. sp. et* M. parvus *Shulman, 1962.* Myxobolus goensis *n. sp. se présente sous la forme de plasmodia digitiformes ou arrondies entre les lamelles branchiales. Leurs spores sont ovales en vue frontale, avec une extrémité antérieure effilée, en forme de citron en vue latérale, measurant 9,7 (9,5-10,5) µm de long, 6,6 (6-7,5) µm de large, et 5,2 (5-6) µm d'épaisseur. Les capsules polaires sont piriformes et de tailles inégales. Les plus grandes mesurent 5,3 (4,5-6) µm de long et 2,4 (2-3) µm de large; les plus petites 2,4 (2-3) µm de long et 1,8 (1,5-2) µm de large. Le filament polaire comporte cinq tours de spire alignés perpendiculairement à l'axe longitudinal des grandes capsules polaires. Au niveau des petites capsules polaires, le filament est difficile à observer et comporte apparemment trois enroulements. Les spores sont différentes de celles des autres espèces de* Myxobolus *infectant* M. cephalus *et autres* Mugil *spp. En outre, le présent matériel se distingue également de celui des 204 espèces de* Myxobolus *avec des capsules polaires de tailles variables. Le fait que seuls les spécimens de* M. cephalus *ont été retrouvés infectés, parmi un échantillon de 206 autres spécimens de poissons de 27 espèces différentes, suggère fortement que ce parasite est spécifique de* M. cephalus.

***MOTS CLÉS :*** *Myxosporea,* Myxobolus goensis *n. sp.,* Mugil cephalus, *poisson, Goa, Inde.*

## INTRODUCTION

*Myxobolus* spp. are the most common myxosporea parasitizing fish. Landsberg & Lom (1991) listed a total of 444 species, and since then a great number of species were described all over the world from a wide variety of both marine and freshwater hosts.

Nineteen *Myxobolus* species were described infecting *Mugil cephalus*: *M. achmerovi* Shulman, 1966, *M. branchialis* Markevitch, 1932, *M. bizerti* Bahri & Marques, 1996, *M. cephalus* (*Myxosoma cephalis* Iversen, Chitty & Van Meter, 1971) Landsberg & Lom, 1991, *M. cheni* Shulman, 1962, *M. chiungchowensis* Chen, 1998, *M. episquamalis* Egusa, Maeno & Sorimachi, 1990, *M. exiguus* Thélohan, 1895, *M. goreensis* Fall, Kpatcha, Diebakate, Faye & Toguebaye, 1997, *M. hannensis* Fall, Kpatcha, Diebakate, Faye & Toguebaye, 1997, *M. ichkeulensis* Bahri & Marques, 1996, *M. mugcephalus* (*Myxosoma microspora* Narasimhamurti, Kalavati & Saratchandra, 1980) Landsberg & Lom, 1991, *M. mugilii* Haldar, Samal & Mukhopadhyay, 1996, *M. mugilis* Negm-Eldin, Govedich & Davies, 1998, *M. mülleri* Bütschli, 1881, *M. parvus* Shulman, 1962, *M. raibauti* Fall, Kpatcha, Diebakate, Faye & Toguebaye, 1997, *M. rohdei* Lom & Dyková, 1994, and *M. spinacurvatura* Maeno, Sorimachi, Ogawa & Egusa, 1990.

At least some of these species are supposed to be both host and tissue specific. The consideration of *M. ach-*

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*merovi* as a parasite of *M. cephalus* may be questioned. This species was first found by Akhmerov (1960) who identified it as *M. oviformis* Thélohan, 1892 as parasite of the Amur wild carp, *Cyprinus carpio haematopterus*. Shulman (1966) reclassified it as a new species (*M. achmerovi*) infecting also the hosts *M. cephalus* and *M. soiyu*. However, the taxonomic distance between carps and mugilids, and the host and tissue specificity observed for some of the *Myxobolus* spp. parasites of *M. cephalus* (Bahri & Marques, 1996), justify the doubts about *M. achmerovi* being a parasite of *M. cephalus*.

Recently the authors had the opportunity of sampling myxosporean parasites of a total of 206 fish specimens, belonging to 27 species, captured at Goa, India. The study of the samples provided the observation of *M. parvus* Shulman, 1962, and another *Myxobolus* species which is described in this paper as *Myxobolus goensis* n. sp., both species parasitizing the gills of *Mugil cephalus*.

## MATERIALS AND METHODS

A total of 206 fish specimens, comprising 27 species, were collected at the Indian Ocean coast of Goa, India, in November, 1999, and transported immediately to the laboratory. Some of the specimens, freshly caught, were acquired to fishermen; others were caught in experimental fisheries and transported alive to the laboratory. The sample included *Amblygaster clupeoides* Bleeker, 1849 (6 specimens), *Arius maculatus* Thunberg, 1830 (5), *Carangoides ciliarius* Rupell, 1830 (12), *Coilia dussumieri* Valenciennes, 1848 (5), *Eleutheronema tetradactylum* Shaw, 1804 (1), *Epinephelus diacanthus* Valenciennes, 1828 (25), *Etroplus suratensis* Bloch, 1790 (1), *Gerres filamentosus* Cuvier, 1829 (6), *Gerres* sp. (6), *Johnius dussumieri* Cuvier, 1829 (4), *Scomberomorus cavalla* Cuvier, 1832 (7), *Lactarius lactarius* Bloch & Schneider, 1801 (7), *Leiognathus equulus* Forsskäl, 1775 (6), *Lutjanus argentimaculatus* Forsskäl, 1775 (3), *Magalaspis cordyla* Linnaeus, 1758 (13), *Mugil cephalus* Linnaeus, 1758 (19), *Nemipterus japanicus* Bloch, 1791 (3), *Parastromateus niger* Bloch, 1795 (10), *Rastralliger kanagurta* Lesson, 1829 (8), *Sardinella longiceps* Valenciennes, 1847 (4), *S. fimbriata* Valenciennes, 1847 (5), *Sciaena aneus* Bloch, 1793 (17), *Scoliodon laticaudatus* Müller & Henle, 1839 (1), *Sillago indica* McKay, Dutt & Sujatha, 1985 (6), *Sphyraena jello* Cuvier, 1829 (8), *Tenulosa ilisha* Hamilton, 1822 (15) and *T. toli* Valenciennes, 1847 (3).

The gills of the specimens were excised, fixed in formalin, and examined latter at the stereomicroscope. Infected gills were routinely processed for histology, and stained with Haematoxylin and Eosin, and Masson's trichrome.

A second sampling was done in November 2003. At this time 37 specimens of *M. cephalus* (total length: 12.5 cm-27.6 cm) were caught and transported alive to the laboratory. The total length of fish from the first sample was not recorded, but the size of the specimens was more or less identical to the second sampling. At the laboratory fish were dissected and all the organs were thoroughly inspected for parasites. Spore measurements was made from 30 fresh spores. For observation of the nature of iodinophilous vacuole, fresh spores were treated with Lugol's iodine solution. Spores were also stained with India Ink for revealing any mucous envelope (Lom & Vávra, 1961).

## RESULTS

### *Myxobolus parvus* Shulman, 1962

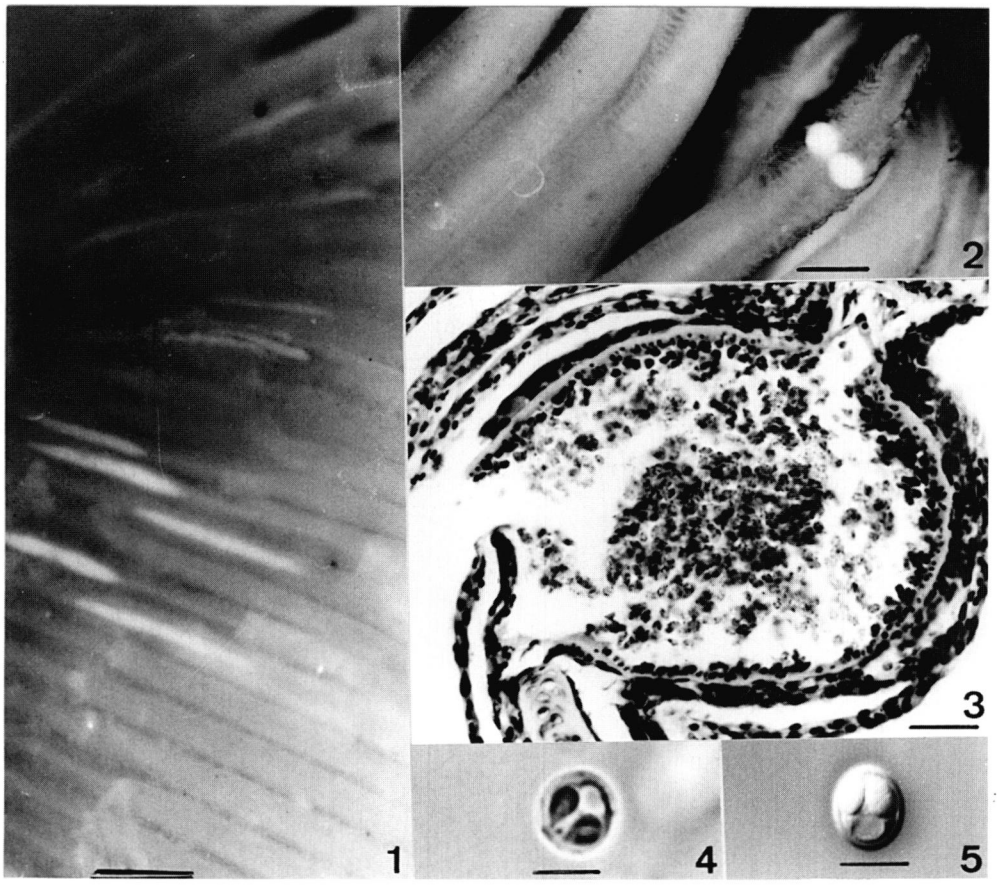
Type host: stripped mullet *Mugil cephalus* L., 1758.
Locality: Goa, India.
Site of infection: gill lamellae.
Prevalence: 29 out from 56 fish (51.8 %) were infected.

Long and thin plasmodia (40-50 × 640-820 µm) with pointed tips were observed in a longitudinal position along the secondary lamellae, appearing as thin, white longitudinal cords in the gills (Fig. 1). Slightly oval (40-100 × 50-240 µm ) or pear shaped (30-40 × 40-80 µm) plasmodia were located in the secondary gill lamellae, near their extremities or in the intermediate positions (Fig. 2).

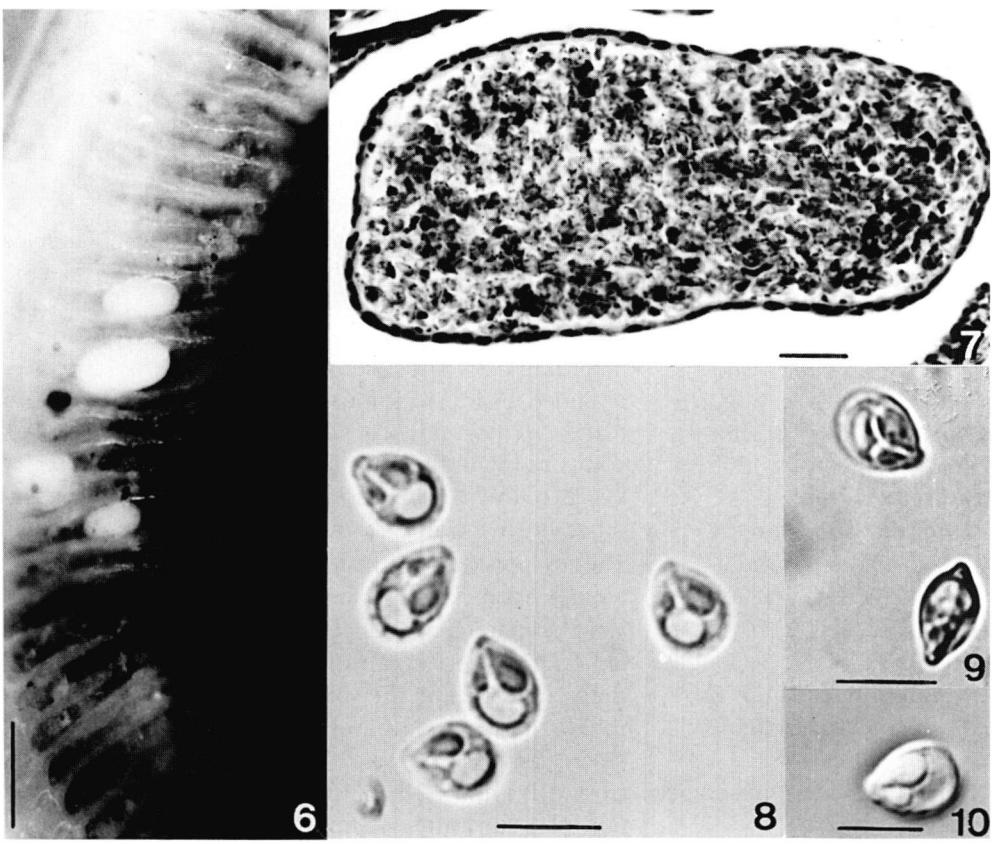
Histologically the plasmodia were surrounded externally by a 5.1-8.3 µm thick layer of epithelial cells. Following this layer there was a thin membrane (about 0.5 µm thick), apparently of parasite origin, well discernible in the sections stained with Masson's trichrome. Internally the plasmodia were coated by an undifferentiated matrix, 3.4-6.8 µm thick. Within the matrix, and on its surface, initial stages of sporogonic development were observed, constituted by small cells with a low amount of cytoplasm. The cells at the periphery of the plasmodia were bigger, and the centre of the plasmodia was full of mature spores (Fig. 3). In other cases, the plasmodia were completely full of mature spores.

Description of the species

The spores (Figs 4 and 5) were round or slightly oval in some cases, in frontal view, and oval in lateral view. The spore valves were relatively thin, symmetrical and smooth. The polar capsules were slightly pyriform, equal in size, and their posterior end reached or sur-




Figs 1-5. – *Myxobolus parvus* Shulman, 1962. 1 - elongate and thin plasmodia in longitudinal position along the secondary gill lamellae (bar = 0.5 mm); 2 - two oval plasmodia (bar = 0.1 mm); 3 - histological section of plasmodia with mature spores in the centre and developmental stages at the periphery (haematoxylin and eosin; bar = 10 µm); 4 - spore (bar = 5 µm); 5 - spore observed under Nomarski phase contrast (bar = 5 µm).




Figs 6-10. – *Myxobolus goensis* n. sp. 6 - plasmodia between gill rakers (bar = 0.2 mm); 7 - histological section of a plasmodia full of mature spores (haematoxylin and eosin; bar = 10 µm); 8 - spores (note the different size of the polar capsules and, in one of the specimens, the sutural marks; bar = 10 µm); 9 - spores (note the lemon-shaped form of the specimen placed in lateral view; bar = 10 µm); 10 - spore observed under Nomarski phase contrast (bar = 10 µm).

passed the midpoint of the spore length. The polar filament was difficult to observe and, apparently, formed 6-7 coils. No intercapsular appendix was present. The spores were 6.3 (6-7) µm long and 4.8 (4.5-6) µm wide, and 4 µm thick. The polar capsules were 3.6 (3-4) µm long and 1.6 (1.5-2) µm wide. There was not a mucous envelope, and a distinct iodinophilous vacuole was observed.

*MYXOBOLUS GOENSIS* N. SP.

Type host: stripped mullet *Mugil cephalus* L., 1758.
Locality: Goa, India.
Site of infection: between gill rakers.
Prevalence: 9 out from 56 (16.0 %) fishes were infected.
Type material: spores have been deposited in the protozoological collection of the Section of Animal Pathology of the Department of Zoology and Anthropology from Faculty of Sciences of Porto, Portugal.

Digitiform cylindrical plasmodia, with rounded extremities (80-120 × 280-360 µm) or, less frequently, round or slightly oval plasmodia (80-120 × 200-240 µm) were located between the gill rakers (Fig. 6). Histologically the plasmodia were enveloped by a thin layer of epithelial cells. Following this layer there was a thin membrane (about 0.5 µm thick) apparently of parasite origin, well discernible in the sections stained with Massson's trichrome. The plasmodia were in different stages of maturity containing only mature spores (Fig. 7), or mature spores in the centre and developmental stages at the periphery.

Description of the species
The spores (Figs 8-11) were oval in frontal view, with tapered anterior extremity and round posterior extremity, and lemon-shaped in lateral view, presenting six sutural marks along the sutural edge. The spore valves were relatively thin, symmetrical and smooth. The polar capsules were markedly unequal in size. They were pyriform, close to each other, and converging in the anterior extremity. The bigger ones could reach, or surpass, the midpoint of the spore length, and the polar filament formed five turns aligned perpendicularly to the longitudinal axis of the spore. Within the smaller polar capsules the polar filament was difficult to observe and, apparently, formed three coils. No intercapsular appendix was present. The spores were 9.7 (9.5-10.5) µm in length, 6.6 (6-7.5) µm in width and 5.2 (5-6) µm in thickness. The larger polar capsules were 5.3 (4.5-6) µm in length and 2.4 (2-3) µm in width. The smaller ones were 2.4 (2-3) µm in length and 1.8 (1.5-2) µm in width. A thin mucous envelope covered the spore surface. A distinct and large iodinophilous vacuole was present.
Concurrent infections by both species were observed in four host specimens.

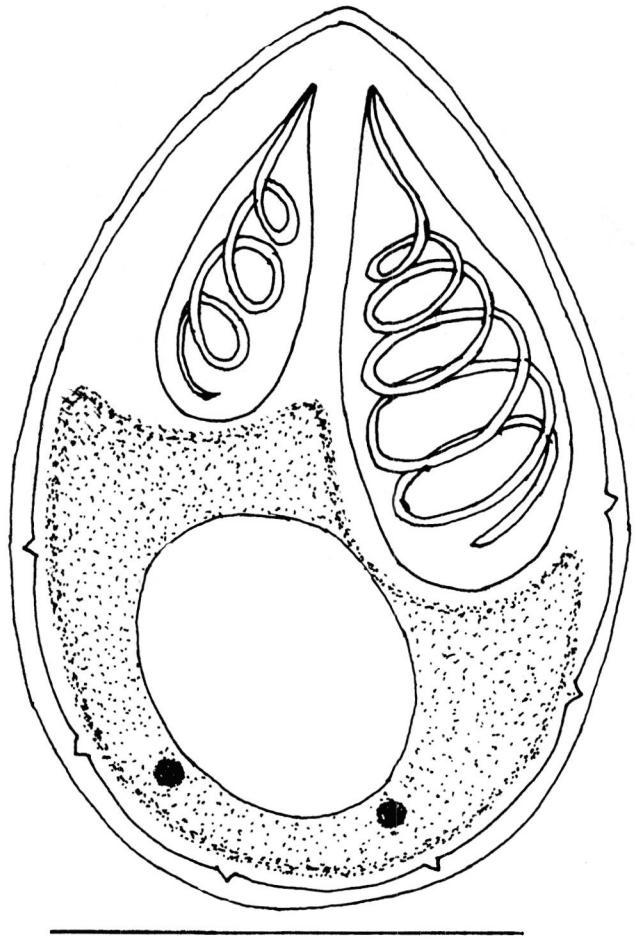


Fig. 11. – Schematic illustration of *Myxobolus goensis* n. sp. (bar = 5 µm).

## DISCUSSION

Our results allow to conclude that both species we examined could be considered specific parasites of *Mugil cephalus*. The fact that only this host species was infected within a sample survey including 187 specimens of other 26 fish species, obtained from the same sampling site, can not be a matter of coincidence. Therefore, our material was compared with the *Myxobolus* species described so far for *M. cephalus*, either located in the gills either in the other organs (Table I).
The spores obtained from the gill lamellae plasmodia are morphometrically quite similar with those of *M. parvus* Shulman, 1962, concerning the size of the spores, although the polar capsules are a little smaller. Other differences exist in the shape of the spores (ellipsoidal in *M. parvus* and round in our material, in frontal view), and in the form and size of the plasmodia (round 1-3 mm cysts in *M. parvus*). However, the location of both forms is similar. Taking into account all

these observations, and in order to prevent the possible creation of synonyms, we consider our material identical to *M. parvus* Shulman, 1962.

The spores from the plasmodia located between the gill rakers seem to be different from those belonging to all the other *Myxobolus* species described so far for *M. cephalus* (Table I). The spore size is distinctly smaller than those of *M. achmerovi*, *M. bizerti*, *M. cephalus*, *M. chiungchowensis*, *M. goreensis*, *M. ichkeulensis*, *M. spinacurvatura*, *M. hannensis* and *M. raibauti*, and bigger than those of *M. parvus*, *M. mugcephalus*, *M. exiguus*, *M. mugiliis*, *M. mugili*, *M. episquamalis*, *M. cheni*, and *M. branchialis*. On the other hand, their length is similar to the spores of *M. mülleri* and *M. rohdei*, but these species have spores much more wider.

Furthermore, the spores from our specimens are completely different from all the other *Myxobolus* species infecting *M. cephalus* due to the presence of polar capsules unequal in size. Moreover, the parasites developed plasmodia located between the gill rakers. All the other *Myxobolus* spp. described from the gills of *M. cephalus* do not have a similar location.

Our specimens were also compared with *Myxobolus* spp. infecting other *Mugil* spp: *M. dasguptai* infecting *M. tade* from India (Haldar *et al.*, 1996), *M. bani* parasitizing *M. curema* from Senegal (Faye *et al.*, 1999), *M. mugchelo* (*Myxobolus mugilis* Parenzan, 1966) Landsberg & Lom, 1991 described for *M. chelo* from Italy (Parenzan, 1966), *M. narasii* (*Myxosoma intestinalis* Narasimhamurti, 1970) Landsberg & Lom, 1991 parasitizing *M. waigensis* from India (Narasimhamurti, 1970), and *M. parenzani* (*Myxobolus branchialis* Parenzan, 1966) Landsberg & Lom, 1991 infecting *M. chelo* from Italy (Parenzan, 1966). These species are quite different from our specimens in having polar capsules of the same size.

Moreover, our material was compared to a total of 739 *Myxobolus* species including nearly all the known species of *Myxobolus*. From these, 204 have differently sized polar capsules. The comparison of their charac-

| | | Spore | | Polar capsule | | |
|---|---|---|---|---|---|---|
| | **Site of infection** | **Length** | **Width** | **Length** | **Width** | **Reference** |
| *M. achmerovi* Shulman, 1966 | Mesentery | 12-14 | 9-10 | 4-5.3 | 2.3-3.5 | Shulman, 1966 |
| *M. bizerti* Bahri & Marques, 1996 | Gill lamellae | 14-14.5 | 6-7 | 5.5-6 | | Bahri & Marques, 1996 |
| *M. branchialis* Markevitch, 1932 | Gill lamellae | 8-7.6 | 6.8-8.5 | 4.4-4.8 | 2.5-4.1 | Bahri & Marques, 1996 |
| *M. cephalus* (*Myxosoma cephalis* Iversen, Chitty & Van Meter, 1971) Landsberg & Lom, 1991 | Brain meninges, gills, mouth, jaw bones | 14-15 | 10-11 | 4-5 | 3-4 | Iversen *et al.*, 1971<br>Landsberg & Lom, 1991 |
| *M. cheni* Shulman, 1962 | Muscles, fins | 8-8.5 | 6-6.5 | 4.5-5 | 2 | Bykhovskaya-Pavlovskaya *et al.*, 1962 |
| *M. chiungchowensis* Chen, 1998 | Intestine | 10.2-11.8 | 9.6-11 | 5.6-6.2 | 3.4-3.8 | Chen & Ma, 1998 |
| *M. episquamalis* Egusa, Maeno & Sorimachi, 1990 | Scales | 7.5-9.5 | 6-7.5 | 3.8-5 | 2-3 | Egusa *et al.*, 1990 |
| *M. exiguus* Thélohan, 1895 | Gill lamellae, pyloric cecae, stomach tissue | 8-9.5 | 6-7.5 | 3-4.5 | 1.5-3 | Bahri & Marques, 1996 |
| *M. goreensis* Fall, Kpatcha, Diebakate, Faye & Toguebaye, 1997 | Gill lamellae | 10-13 | 10-13 | 4-5 | 2-4 | Fall *et al.*, 1997 |
| *M. hannensis* Fall, Kpatcha, Diebakate, Faye & Toguebaye, 1997 | Gill lamellae, gill arches | 13-15 | | 7-9 | 5-6 | Fall *et al.*, 1997 |
| *M. ichkeulensis* Bahri & Marques, 1996 | Gill arches | 13-14 | 12-13 | 5-6 | 4-4.3 | Bahri & Marques, 1996 |
| *M. mugcephalus* (*Myxosoma microspora* Narasimhamurti, Kalavati & Saratchandra) Landsberg & Lom, 1991 | Gill lamellae | 4.8-5.2 | | 1.6-2.0 | 1.0-1.2 | Narasimhamurti *et al.*, 1980<br>Landsberg & Lom, 1991 |
| *M. mugilii* Haldar, Samal & Mukhopadhyay, 1996 | Gill lamellae | 8.1-16.3 | 4.0-7.3 | 2.4-8.1 | 1.6-4.0 | Haldar *et al.*, 1996 |
| *M. mugilis* Negm-Eldin, Govedich & Davies, 1999 | Gill lamellae | 7.4 | 7.3 | 3.6<br>2.4 | 2.1<br>1.2 | Negm-Eldin *et al.*, 1999 |
| *M. mülleri* Bütschli, 1881 | Gill lamellae | 10-12 | 9-11 | 4-5 | 2-3 | Bahri & Marques, 1996 |
| *M. parvus* Shulman, 1962 | Gill lamellae | 6.5-7 | 5.5-6 | 3.8-4.2 | 2 | Bykhovskaya-Pavlovskaya *et al.*, 1962 |
| *M. raibauti* Fall, Kpatcha, Diebakate, Faye & Toguebaye, 1997 | Liver | 14-16 | 12-13 | 5-6.5 | 3-4 | Fall *et al.*, 1997 |
| *M. rohdei* Lom & Dyková, 1994 | Kidney | 9.8-11.6 | 8.4-9.1 | 3.7-5 | 2.5-3.1 | Lom & Dyková, 1994 |
| *M. spinacurvatura* Maeno, Sorimachi, Ogawa & Egusa, 1990 | Mesentery, brain | 10.5-12.5 | 9-11 | 3.5-5 | 2.5-3.5 | Maeno *et al.*, 1990 |
| *M. parvus* Shulman, 1962 | Gill lamellae | 6-7 | 4.5-6 | 3-4 | 1.5-2 | This paper |
| *M. goensis* n. sp. | Gill rakers | 9.5-10.5 | 6-7.5 | 4.5-6<br>2-3 | 2-3<br>1.5-2 | This paper |

Table I. – Characteristics of the *Myxobolus* species infecting *Mugil cephalus*.

teristics showed that they do not conform with the features we observed in the specimens from Goa.
Taking into account the characteristics of the specimens and the location of the parasites in the gill rakers, it seems justifiable to consider the present material as belonging to a new species, and we propose the name *Myxobolus goensis* n. sp.

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