Systematic Parasitology **32:** 125–129, 1995.

A new myxosporean, *Zschokkella leptatherinae* n. sp. (Myxozoa: Myxidiidae), from the hepatic ducts and gall-bladder of Australian marine fishes

Xiao-qun Su and R.W.G. White
Department of Zoology, University of Tasmania, GPO Box 252C, Hobart, Tasmania 7001, Australia

Accepted for publication 25th August, 1994

Abstract

A new myxosporean, *Zschokkella leptatherinae* n. sp., was found in the hepatic ducts and gall bladder of five atherinid fish species, *Leptatherina presbyteroides*, *Atherinosoma microstoma*, *Kestratherina brevirostris*, *K. esox* and *K. hepsetoides*, collected at Dru Point, Margate in south-eastern Tasmania, Australia. This species has a round, ellipsoid or irregular plasmodium. The plasmodium is enclosed by a surface membrane and the cytoplasm is composed of an outer homogeneous ectoplasm and an inner coarse endoplasm with large clear areas, numerous vacuoles and spores which differentiate in the central area of the endoplasm. The characteristic metrical data of the myxosporean are as follows: spores 15.3 × 11.8 μm; polar capsules 3.9 × 3.4 μm. This is the third *Zschokkella* species reported from Australia.

Introduction

Although nearly 40 species of *Zschokkella* Auerbach, 1910 have been reported from around the world (e.g. Institute of Hydrobiology Academia Sinica, 1973; Yoshino & Noble, 1973; Kovaljova & Gaevskaya, 1982; Chen & Hsieh, 1984; Gaevskaya *et al.* 1985; Sarkar, 1987; Wierzbicka, 1987; Diamant & Paperna, 1992; Sitjà-Bobadilla & Alvarez-Pellitero, 1993), only two have been recorded in Australia (Moser *et al.*, 1989; Su & White, in press). During our studies of protozoan parasites of marine fishes in south-eastern Tasmania, Australia, a new species of *Zschokkella* was detected from the hepatic ducts and gall-bladder of five atherinid fishes, *Leptatherina presbyteroides* (Richardson), *Atherinosoma microstoma* (Günther), *Kestratherina brevirostris* Pavlov Ivanstoff, Last & Cowley, *K. esox* (Klunzinger) and *K. hepsetoides* (Richardson). The description of this new species and the prevalence of infection are reported in this paper.

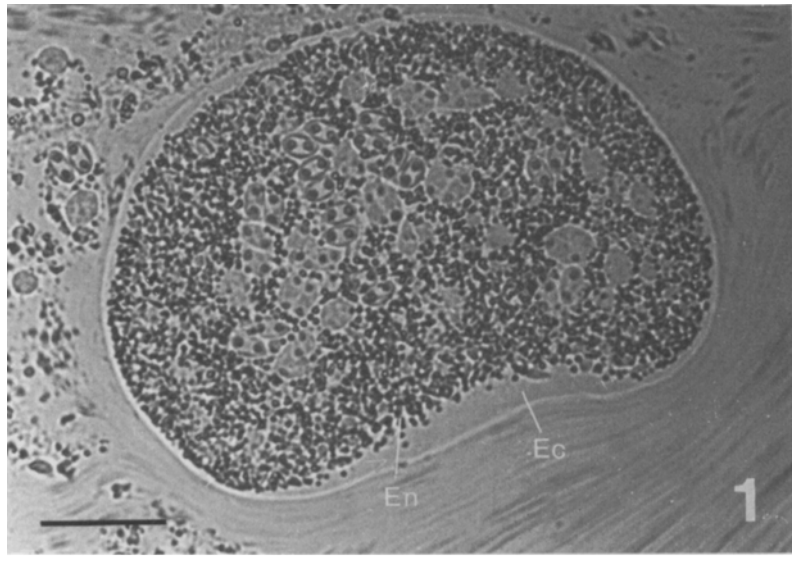
Materials and methods

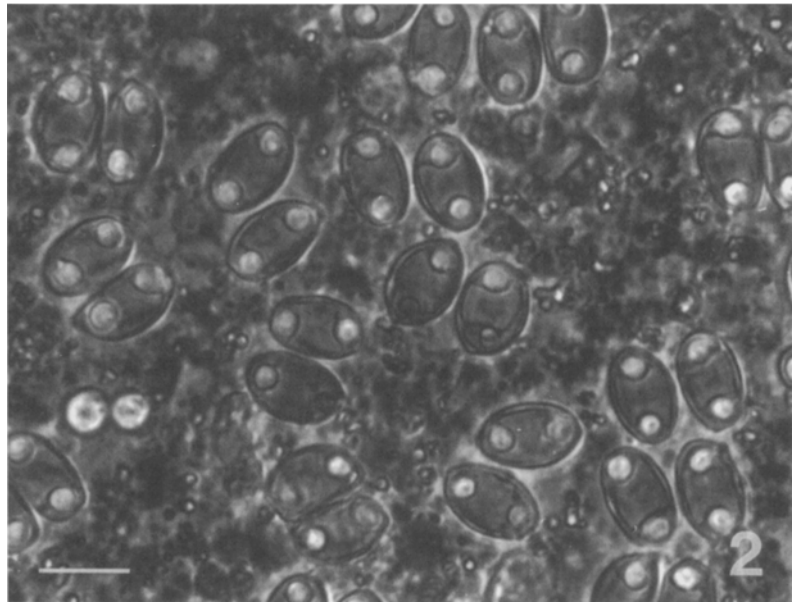
Collection of materials

All the materials for this study were collected from seagrass meadows at Dru Point, about 40 km south-east of Hobart, Tasmania, Australia from December 1989 to June 1992. Robertson & White (1985) gave a full description of the site and the fish collecting methods. Myxosporean was obtained from the liver and gall-bladder immediately after the fish were killed and dissected.

Light microscopy

Myxosporean spores were examined from fresh preparations of liver tissue with a Zeiss Standard Universal microscope equipped with phase contrast and a Zeiss Axiovert 35 microscope with a × 100 differential interference contrast objective. All measurements were made on 50 fresh specimens and are given in micrometres (μm) followed by averages in parentheses. The figures were drawn with the aid of a camera lucida. Photomicrographs were taken on ILFORD FP4 film. Holotype specimens are deposited in the Tasmanian Museum and Art Gallery (TMAG), Argyle Street, Hobart, Tasmania 7000, Australia.





Figs 1–2. 1. Photomicrograph of plasmodium of *Zschokkella leptartherinae* n. sp. *Abbreviations*: Ec, ectoplasm; En, endoplasm. *Scale bar*: 50 μm. 2. Photomicrograph of fresh spores of *Zchokkella leptatherinae* n. sp.; note that spores usually occur in pairs. *Scale-bar*: 10 μm.

Scanning electron microscopy

Infected liver tissue pieces (2–4 mm) were fixed in a solution of 3% glutaraldehyde with 0.1 M phosphate buffer (pH 7.2) for 60 min; the tissue was then rinsed in the buffer solution. Post-fixation was for 60 min in 1% osmium tetroxide solution in 0.1 M phosphate buffer (pH 7.2); it was again rinsed in the buffer. Dehydration was carried out in graded acetone and final drying was achieved in a Balzers Union CPD 020 critical-point drier. The tissue was coated with ultra-pure gold in a Balzers Union sputter coater to give a film thickness of 25 nm. The specimens were observed with a Phillips 505 scanning electron microscope at accelerating volt-

age of 15 kV and a spot size of 20 nm. Images were recorded with Polaroid 667 film.

Statistical method

The statistical analysis was conducted using Statview 512 software on a Macintosh computer. Variation in the prevalence of myxosporean infection in different fish species was tested using a 2 × c contingency table. A significant level of 99% ($p < 0.01$) was taken as a rejection of the null hypothesis which states that there was no dependence between the variables.

Zschokkella leptatherinae n. sp.

Type-host: *Leptatherina presbyteroides*.
Other hosts: *Atherinosoma microstoma*, *Kestratherina brevirostris*, *K. esox* and *K. hepsetoides*.
Type-locality: Dru Point, Margate, Tasmania, Australia.
Sites: Hepatic ducts and gall-bladder.
Type-specimens: Holotype no. TMAG-K1310, from *L. presbyteroides* is deposited in the TMAG; paratypes no. 300367, from *A. microstoma*; no. 400038, from *K. esox* and no. 100061 from *K. brevirostris* are in the collection of senior author.

Description

Vegetative form. There are numerous plasmodia in the hepatic ducts of the liver of infected atherinid fish examined under the light microscope; they are rounded, ellipsoid or irregular (Fig. 1). The plasmodia vary in size with the development of internal spores. Plasmodia without mature spores are slightly smaller (9–473 μm across) than plasmodia containing mature spores (38–520 μm across). Occasionally, large plasmodia are visible with the naked eye. There are 7–c.1,000 spores in one plasmodium. The plasmodium has a surface membrane and the cytoplasm is composed of an outer ectoplasm and an endoplasm. The outer ectoplasm presents as a thin clear homogeneous layer, whilst the endoplasm is coarse, basically dark grey with large clear areas and numerous yellow-brown vacuoles. Sporoblasts, in different developmental stages, are present in the endoplasm. Mature spores are found near the centre of the plasmodium. Two spores are usually together, suggesting an origin in disporic pansporoblasts (Fig. 2).

Spores. Mature spores are oval or ellipsoid in both valvular and sutural views, with slightly pointed ends when observed under an oil immersion light microscope (Figs 3,4); they vary in length from 13.0–17.0 (15.3) μm, width from 9.5–14.0 (11.8) μm and thickness from 9.0–13.0 (11.1) μm. Both spore valves are vaulted and moderately thick. They meet meridionally to form a slightly curved sutural ridge which is approximately 1.5 μm wide. Ten to 12 slightly curved longitudinal striations are visible on the spore valve (Fig. 3). These striations are seen more clearly under the scanning electron microscope (Fig. 5). The pointed ends of spore are not evident under the scanning electron microscope. The polar capsules are almost oval with slightly attenuated distal ends. They are situated symmetrically at opposite poles of the spore, opening at some distance from the end of the spore and both to one side. They are equal in size, 3.5–5.0 (3.9) μm in length and 3.0–4.0 (3.4) μm in width. The polar filament is regularly coiled into a pyriform spiral, usually with 4–5 coils which lie perpendicular to the long axis of the capsule (Fig. 4). When extruded, the length of the polar filament measures 18.0–34.0 (22.5) μm in the stained specimen. A large sporoplasm lies between the polar capsules; among it are 2 small spherical nuclei measuring 1.0–1.5 μm. Two nuclei of capsulogenic cells lie on the periphery of the polar capsules (Fig. 4).

Prevalence of infection

All 5 atherihid species sampled from Tasmanian waters, namely *Leptatherina presbyteroides*, *Atherinosoma microstoma*, *Kestratherina brevirostris*, *K. esox* and *K. hepsetoides*, were infected by *Zschokkella leptatherinae* n. sp. Another 6 fish species occurring sympatrically with these atherinids were not infected. The prevalence in different hosts and different sites is shown in Table I.

There is a significant variation in the prevalence of infection among fish species (2 × c contingency table analysis, $\chi^2 = 72.353$, $p < 0.001$). Except for *K. hepsetoides* (only 5 individuals were examined), *Atherinosoma microscoma* and especially *Leptatherina presbyteroides* appear to be the preferred host species for *Z. leptatherinae*.

Z. leptatherinae also exhibits an obligate organ specificity. It was found only in the hepatic ducts and gall-bladder of atherinid fish. No spores were observed in the intestine, kidney, blood, spleen, reproductive organs and adipose tissue. Not every liver-infected

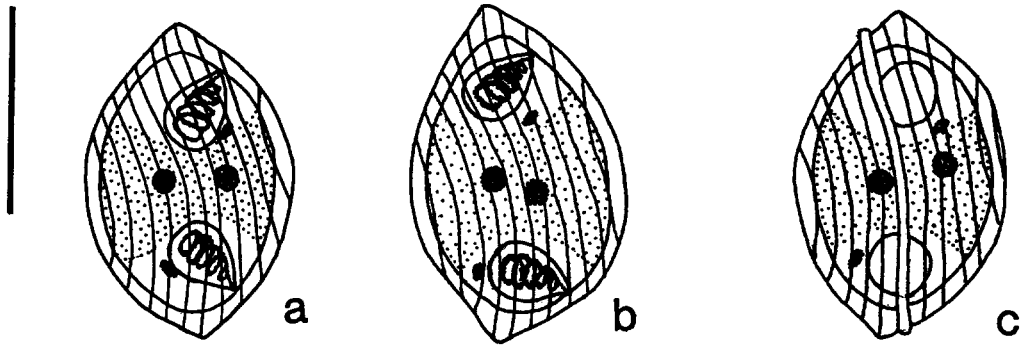


Fig. 3. Diagrams of spores of *Zschokkella leptatherinae* n. sp.: a–b, valvular view; c, sutural view. *Scale-bar*: 10 μm.

Table 1. Prevalence of *Zschokkella leptatherinae* n. sp. infection in different fish species and sites.

Fish species	Prevalence in hepatic ducts (%)	Prevalence in gall-bladder (%)	No. of fish examined
Leptatherina presbyteroides	31.9	18.3	589
Atherinosoma microstoma	16.3	5.6	514
Kestratherina esox	6.3	3.1	64
Kestratherina brevirostris	5.2	2.0	116
Kestratherina hepsetoides	40.4	20.0	5

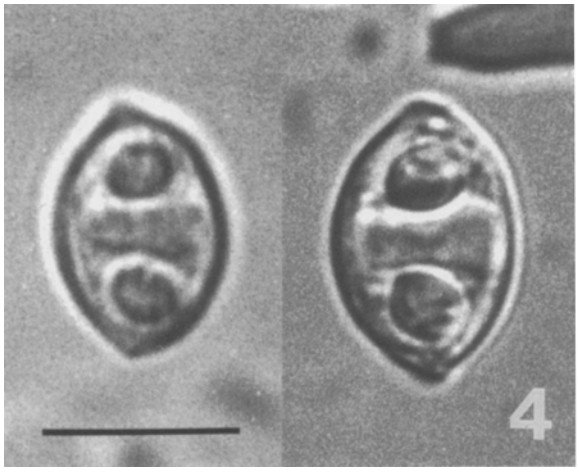


Fig. 4. Interference contrast photomicrographs of fresh spores of *Zschokkella leptatherinae* n. sp.; note the pointed ends of the spores. *Scale-bar*: 10 μm.

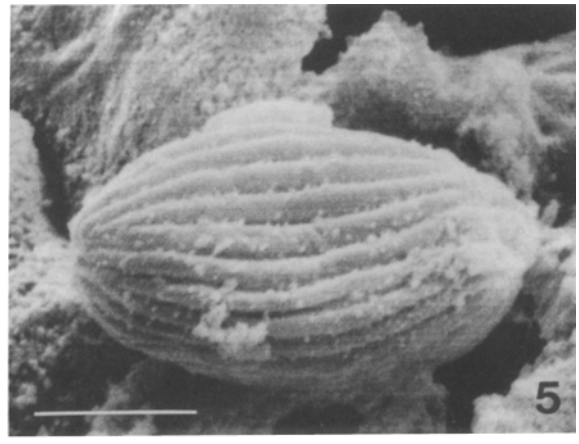


Fig. 5. Scanning electron micrograph of spore of *Zschokkella leptatherinae* n. sp. *Scale-bar*: 5 μm.

fish has gall-bladder infection, but all fish with gall-bladder infection have liver infection. This suggests that the gall-bladder might be the secondary site for this parasite.

Discussion

The identification of myxosporeans has previously been mainly based on the spore features and the host and/or tissues they have infected. Two *Zschokkella* spp. have been previously reported from Australian marine fishes (Moser *et al.*, 1989; Su & White, in press): *Z. heronensis* Moser *et al.*, 1989 from the gall-bladder of *Chaetodon plebeius* and *Choerodon*

venustus on the Great Barrier Reef, Queensland and *Z. macrocapsula* Su & White, in press, from the gall-bladder of *Leptatherina presbyteroides* and *Atherinosoma microstoma* at Dru Point, Tasmania. *Z. leptatherinae* n. sp. differs markedly from *Z. heronensis* in the position and opening of the polar capsules and the dimension of the spore. It can also be distinguished from *Z. macrocapsula* in having longitudinal striations on the spore valves, smaller polar capsules and fewer coils of polar filament. Comparing *Z. leptatherinae* n. sp. with other previously described species from elsewhere, there are two species which show close resemblance to the present species: *Z. orientalis* Konovajov & Schulman, 1966 from the gall-bladder of *Oncorhynchus gorbuscha*, *O. kisutch*, *O. mykiss*, *O. tschawytscha*, *Salvelinus* and *S. leuomens* from the former USSR (Schulman, 1966) and *Z. russelli* Tripathi, 1948 from the gall-bladder and hepatic ducts of *Gaidrpsarus mediterraneus* and *Ciliata mustela* from off the coasts of Britain and North America (Tripathi, 1948; Davies, 1985). However, the spores of *Z. orientalis* are consistently smaller than those of the present species. Although morphologically similar, *Z. leptatherinae* n. sp. can be distinguished from *Z. russelli* in the following characteristics: the spores of *Z. leptatherinae* are oval or ellipsoid in valvular view and have narrow pointed ends, whilst the spores of *Z. russelli* are gibbous in valvular view and have rounded ends; the spore valves of *Z. leptatherinae* have 10–12 slightly curved longitudinal striations, whilst those of *Z. russelli* have 9–18 straight striations; the polar capsules of *Z. leptatherinae* are oval and open at some distance from the end of the spore, whilst those of *Z. russelli* are spherical and open more or less terminally; the extruded polar filaments of *Z. leptatherinae* are 18.0–34.1 μm in length, whilst those of *Z. russelli* are 37–50 μm in length; and the dimensions of the plasmodium of *Z. leptatherinae* are 9–520 μm, whilst those of *Z. russelli* are only 10–180 μm. Finally, it is worth noting that *Z. leptatherinae* seems to have a relatively high host specificity, since, at the study site, it infects only atherinid fishes and six other sympatric fish species were not infected. For these reasons, we consider *Z. leptatherinae* to be a new species.

Acknowledgements

We would like to thank Mr W. Jablonski for his assistance with scanning electron microscopy. Thanks are also due to Dr J. Gorst for providing access a light microscope equipped with DIC.

References

Chen, C.L. & Hsieh, S.R. (1984) New species of *Zschokkella* (Myxosporida) from freshwater fishes of China. In: (Institute of Hydrobiology Academia sinica). *Parasitic organisms of freshwater fish of China*. Beijing: Agricultural Publishing House, pp. 82–88.

Davies, A.J. (1985) *Zschokkella russelli* Tripathi (Myxozoa: Myxosporea) from the five-bearded rockling, *Ciliata mustela* L. (Teleostei: Gadidae) in Wales. *Journal of Fish Diseases*, **8**, 299–308.

Diamant, A. & Paperna, I. (1992) *Zschokkella icterica* sp. nov. (Myxozoa, Myxosporea), a pathogen of wild rabbitfish *Siganus iuridus* (Ruppell, 1892) from the Red Sea. *European Journal of Protistology*, **21**, 71–78.

Gaevskaya, A.V., Kovaljova, A.A. & Krasin, V.K. (1985) New species of myxosporidians (Protozoa, Myxosporidia) from urinary bladders of grenadiers in the Atlantic and Pacific. *Zoologicheskii Zhurnal*, **64**, 1576–1579. (In Russian).

Institute of Hydrobiology Academia Sinica (1973) *An illustrated guide to the fish diseases and causative pathogenic fauna and flora in Hubei Province*. Beijing: Scientific Publishing House, pp. 57–92.

Kovaljova, A.A. & Gaevskaya, A.V. (1982) Myxosporidia of the genus *Zschokkella* (Protozoa) from fishes of south-west Atlantic. *Zoologicheskii Zhurnal*, **61**, 933–935. (In Russian).

Moser, M., Kent, M.L. & Dennis, D. (1989) Gall bladder Myxosporea in coral reef fishes from Heron Island, Australia. *Australian Journal of Zoology*, **37**, 1–13.

Robertson, C.H. & White, R.W.G. (1985) Feeding patterns of *Nesogobius* sp., *Gymnaspistes marmoratus*, *Neoodax balteatus* and *Acanthaluteres spilomelanurus* from a Tasmanian seagrass meadow. *Australian Journal of Marine and Freshwater Research*, **37**, 481–489.

Sarkar, N.K. (1987) Studies on myxosporidian parasites (Myxozoa: Myxosporea) from marine fishes in west Bengal, India. 2. Description of two new species from *Tachysurus platystomus* (Day). *Archiv für Protistenkunde*, **133**, 151–155.

Schulman, S.S. (1966) [*Myxosporidia of the USSR*.] Moscow-Leningrad: Nauka, pp. 145–149 (In Russian: English translation, S. Sharma, 1990. Rotterdam: A. A. Balkema).

Sitjà-Bobadilla, A. & Alvarez-Pellitero, P. (1993) *Zschokkella mugilis* n. sp. (Myxosporea: Bivalvulida) from Mullets (Teleostei: Mugilidae) of Mediterranean Waters: light and electron microscopic description. *Journal of Eukaryotic Microbiology*, **40**, 755–764.

Su, X. & White, R.W.G. New myxosporeans (Myxozoa: Myxosporea) from marine fishes of Tasmania, Australia. *Acta Protozoologica* (in press).

Tripathi, Y.R. (1948) Some new Myxosporidia from Plymouth with a proposed new classification of the order. *Parasitology*, **39**, 110–118.

Wierzbicka, J. (1987) *Zschokkella stettinensis* sp. n. (Myxospora, Bivalvulida) – a parasite of eel, *Anguilla anguilla* (L). *Acta Protozoologica*, **26**, 79–82.

Yoshino, T.P. & Noble, E.R. (1973) Myxosporida in macrourid fishes of the North Atlantic. *Canadian Journal of Zoology*, **51**, 745–752.