Morphometric and molecular characterisation of four new *Ceratomyxa* species (Myxosporea: Bivalvulida: Ceratomyxidae) from fishes off Lizard Island, Australia

Nicole L. Gunter^{1,2,3}, Mieke A.A. Burger^{1,2} and Robert D. Adlard^{1,2}

¹Biodiversity Program, Queensland Museum, P.O. Box 3300, South Brisbane, Queensland 4101, Australia;

²School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland 4072, Australia;

³Australian National Insect Collection, Commonwealth Scientific and Industrial Research Organisation Entomology, Black Mountain Laboratories, P.O. Box 1700, Canberra, ACT 2601, Australia

Abstract: Four new species of *Ceratomyxa* Thélohan, 1892 are described from the gall bladders of fishes collected off Lizard Island, Australia. These species are characterised using a combination of morphometric and molecular data. *Ceratomyxa bartholomewae* sp. n. is described from *Hyporhamphus dussumieri* (Valenciennes) (family Hemirhamphidae); *C. koieae* sp. n. is described from *Sphyræna forsteri* Cuvier (family Sphyrænidae); *C. pantherini* sp. n. is described from *Bothus pantherinus* (Rüppell) (family Bothidae) and *C. reidi* sp. n. is described from *Chaetodon vagabundus* Linnaeus (family Chaetodontidae). A fifth species from *Zebrasoma veliferum* (Bloch) (family Acanthuridae) is also reported but due to limited material is not formally described here.

Keywords: *Ceratomyxa*, Myxosporea, Myxozoa, phylogeny, SSU rDNA, new species

Ceratomyxa Thélohan, 1892 is the second largest myxozoan genus with just over 260 species described globally. To date, 35 species have been described from the gall bladders of fishes collected from the Great Barrier Reef (GBR) Australia with only 10 of these collected off Lizard Island (Moser et al. 1989, Gunter and Adlard 2008, 2009, Heiniger et al. 2008, Gunter et al. 2009). During a parasitological survey of fishes collected off Lizard Island, species of *Ceratomyxa* were found in the gall bladders of *Bothus pantherinus* (Rüppell), *Chaetodon vagabundus* Linnaeus, *Hyporhamphus dussumieri* (Valenciennes), *Sphyræna forsteri* Cuvier, and *Zebrasoma veliferum* (Bloch). All of these species are new to science and four species are described herein.

MATERIALS AND METHODS

Host and parasite collection. Fishes were collected by line, spear-fishing and seine-netting off Lizard Island (14°40'S, 145°27'E) in the northern Great Barrier Reef. Fish were euthanised using neural pithing. The gall bladders were removed from the abdominal mass, placed in a cavity block and ruptured. A drop of bile was placed on a glass microscope slide and covered with a glass cover-slip. The slide was examined using a light microscope at ×400 magnification. Infected samples were preserved in 100% ethanol for DNA analysis and frozen in saline for the measurement of spores.

Morphological analysis of spores. Morphological measurements of spores followed the guidelines devised by Lom and Arthur (1989) for species descriptions of Myxosporea. However, for characterisation of ceratomyxid spores, an additional measurement of the posterior spore angle was made as per Heiniger et al. (2008) and apical width of spores was recorded when possible. If plasmodia were observed, digital images of fresh material were taken in the field. Spores were characterised from frozen material to prevent shrinkage often caused by the use of chemical fixatives. Thirty spores were measured from digital images taken at ×400 magnification using a Nikon Digital Sight DS-L1 (Nikon Corporation, Japan) except for *Ceratomyxa koieae* sp. n. where only 20 mature spores were characterised due to the small number of mature spores in the sample. Apical width measurements were recorded from as many spores as possible ($n = 0-9$). Measurements were calibrated using a micrometer. The measurements are given in micrometres (µm) as the mean with standard deviation, followed by the range and number of measurements (n) in parentheses.

Small Subunit rDNA analysis. DNA of *Ceratomyxa* spp. was extracted from 250 µl of infected bile preserved in ethanol. The sample was pelleted at 4,000 g for 10 min and the ethanol removed. DNA was extracted from the pellet as per the recommended protocol accompanying the QIAgen DNeasy Kit (QIAGEN Inc., Valencia, California). Partial small subunit ribosomal DNA (SSU rDNA) was amplified by PCR using the primers MyxospecF 5' TTC TGC CCT ATC AAC TWG TTG (Fiala 2006) and 18R 5' CTA CGG AAA CCT TGT TAC G (Whipps

et al. 2003). PCR and sequencing reactions were carried out as described by Heiniger et al. (2008).

Phylogenetic analysis. The SSU rDNA region from the taxa sequenced in this study were edited using BioEdit version 7.0.0 (Hall 1999). Selected SSU rDNA sequences (Table 1) were downloaded from GenBank and included one representative of each *Ceratomyxa* species available in GenBank. *Ellipsomyxa gobii* was chosen as the outgroup. An alignment was produced using CLUSTAL W (Thompson et al. 1994), edited by eye and, when trimmed using BioEdit version 7.0.0 (Hall 1999), a 1,664 base alignment was produced. Maximum parsimony (MP) and Neighbour Joining (NJ) analyses were conducted in PAUP* 4.0b 10 (Swofford 2003). MP employed a heuristic search with 10 repetitions of random sequence addition, tree bisection and reconnection branch swapping. NJ analysis was conducted using default parameters of PAUP* 4.0b 10. The strength of the resultant relationships was tested by bootstrap analyses with 1,000 replicates for both MP and NJ analyses. For maximum likelihood (ML), the optimal evolutionary model of nucleotide substitution using Akaike Information Criterion was determined using Modeltest 3.06 (Posada and Crandall 1998) which identified the general time reversible (GTR+I+G) model with proportion of invariable sites = 0.271 and gamma distribution = 0.639 estimated from the data. ML analysis was performed using PAUP* 4.0b 10 with above parameters. Bootstrap confidence values were calculated with 100 replicates. Bayesian analyses (BA) were conducted in MrBayes (Ronquist and Huelsenbeck 2003) using the evolutionary model as above, with 2 million generations and tree sampling every 100 generations. Graphical and statistical analyses implemented in Tracer 1.4 (<http://beast.bio.ed.ac.uk/Tracer>) were used to check the performance and accuracy of MrBayes analysis. Based on the results, the last 10,000 trees were used to build the consensus tree.

RESULTS

Species descriptions

Ceratomyxa bartholomewae sp. n.

Figs. 1A–D, 2A–C, Table 2

Spores (Figs. 1A–D, 2A–C): Mature spore slightly crescent-shaped in lateral view, 5.5 ± 0.3 (4.7–6.2) in length and 17.5 ± 1.8 (13.5–21.7) in thickness ($n = 30$). Cylindrical in apical view, 5.4 ± 0.3 (5.0–5.9) in width ($n = 8$). Posterior angle slightly concave to slightly convex (154.6 – 183.1°). Valves unequal in size, smoothly ovoid in lateral view. Straight suture visible between valves. Spherical polar capsules, 2.3 ± 0.3 (1.8–2.8) in length and 2.3 ± 0.2 (1.6–2.7) in width ($n = 30$), positioned centrally in the apex of spore but occasionally off centre in lateral view, centrally located in apical view. Occasional spores with 3 valves observed (See Fig. 1C).

Plasmodia: Disporous (Fig. 1D).

Type host: *Hyporhamphus dussumieri* (Valenciennes) (Dussumier's halfbeak), Family Hemirhamphidae.

Type locality: Off Lizard Island, Great Barrier Reef, Queensland ($14^\circ 39'S$, $145^\circ 27'E$).

Site of infection: Gall bladder.

Prevalence: 1 of 10 (10%).

Type material: Syntypes – air-dried slides stained with Giemsa, registration numbers G465404–465406, deposited in the collections of the Queensland Museum, Brisbane, Australia.

Etymology: In honour of Jerri Bartholomew, Oregon State University, for her research of *Ceratomyxa shasta*.

Remarks: *Ceratomyxa bartholomewae* is morphologically similar to *Ceratomyxa gibba* Meglitsch, 1960, *C. priacanthi* Kalavati, Dorothy et Pandian, 2002, *C. sprengi* Moser, Kent et Dennis, 1989 and *C. robertsthomsoni* Gunter, Whipps et Adlard, 2009. It can be distinguished from *C. gibba* and *C. priacanthi* on the basis of the shape and size of the polar capsules. *Ceratomyxa bartholomewae* has spherical polar capsules which are smaller ($2.3 \times 2.3 \mu\text{m}$) than *C. priacanthi* ($5.2 \times 2.8 \mu\text{m}$), while the polar capsules of *C. gibba* are pyriform ($2.8 \times 2.3 \mu\text{m}$). *Ceratomyxa gibba* also has longer spores ($6.9 \mu\text{m}$) than *C. bartholomewae* ($5.5 \mu\text{m}$). The spores of *C. sprengi* are generally thinner, but have a greater length range (16.3×4 – $8 \mu\text{m}$) than those of *C. bartholomewae* (17.5×4.7 – $6.2 \mu\text{m}$) and the anterior and posterior margins of *C. sprengi* spores are more or less straight while the posterior angle of *Ceratomyxa bartholomewae* is slightly convex. *Ceratomyxa bartholomewae* has longer spores ($5.5 \mu\text{m}$) than *C. robertsthomsoni* ($4.7 \mu\text{m}$) but can also be distinguished by SSU rRNA sequence data.

A total of 1,403 bases of SSU rDNA was generated for *Ceratomyxa bartholomewae* (GenBank accession GU136391). The sequence differs from the aligned sequences of *Ceratomyxa* spp. at 154–283 of the 1,664 nucleotide alignment and has a maximum genetic similarity of 87.5% with *C. dennisi*.

This is the first record of a *Ceratomyxa* infection in a species from the family Hemirhamphidae (Belontiiformes).

Ceratomyxa koieae sp. n. Figs. 2D–F, 3A, B, Table 2

Spores (Figs. 2D–F, 3A, B): Mature spore slightly crescent-shaped in lateral view, 5.5 ± 0.4 (4.9–6.7) in length and 36.7 ± 3.4 (28.6–41.2) in thickness ($n = 30$). Cylindrical in apical view, 5.3 ± 0.1 (5.1–5.4) in width ($n = 3$). Posterior angle slightly concave to slightly convex (156.3 – 183.8°). Valves almost equal in size, smoothly ovoid in lateral view. Straight suture visible between valves. Subspherical polar capsules, 2.8 ± 0.2 (2.5–2.9) in length and 2.6 ± 0.2 (2.3–2.9) in width ($n = 30$), positioned at apex of spore, centrally located in apical view.

Plasmodia: Not observed.

Type host: *Sphyræna forsteri* Cuvier (bigeye barracuda), Family Sphyrænidae.

Type locality: Off Lizard Island, Great Barrier Reef, Queensland ($14^\circ 39'S$, $145^\circ 27'E$).

Site of infection: Gall bladder.

Prevalence: 1 of 2 (50%).

Type material: Syntypes – air-dried slides stained with Giemsa, registration numbers G465407–465409, deposited

Fig. 1. *Ceratomyxa bartholomewae* sp. n. from *Hyporhamphus dussumieri*; photomicrographs of spores. **A** – sutural view; **B** – apical view; **C** – atypical 3-valved spore; **D** – disporous plasmodium. Scale bar = 10 μ m.

Fig. 2. Diagrammatic illustrations of morphological variability of spores of new *Ceratomyxa* species. **A–C** – *Ceratomyxa bartholomewae* sp. n.; **D–F** – *Ceratomyxa koieae* sp. n.; **G–I** – *Ceratomyxa pantherini* sp. n.; **J–L** – *Ceratomyxa reidi* sp. n.; **M, N** – *Ceratomyxa* sp. ex *Zebrasoma veliferum*.

Fig. 3. *Ceratomyxa koieae* sp. n. from *Sphyræna forsteri*; photomicrographs of spores. **A** – sutural view; **B** – apical view. Scale bar = 10 μ m.

Table 1. Sequences included in molecular analyses.

Species	GenBank Acc. No.	Host	Reference
<i>Ceratomyxa anko</i>	DQ301510	<i>Lophius litulon</i> (Lophiiformes: Lophiidae)	Freeman et al. 2008
<i>Ceratomyxa atkinsoni</i>	FJ204244	<i>Lethrinus atkinsoni</i> (Perciformes: Lethrinidae)	Gunter et al. 2009
<i>Ceratomyxa auerbachii</i>	EU616732	<i>Clupea harengus</i> (Clupeiformes: Clupeidae)	Køie et al. 2008
<i>Ceratomyxa barnesi</i>	FJ204245	<i>Siganus lineatus</i> (Perciformes: Siganidae)	Gunter et al. 2009
<i>Ceratomyxa bartholomewae</i> sp. n.	GU136391	<i>Hyporhamphus dussumieri</i> (Beloniformes: Hemirhamphidae)	This paper
<i>Ceratomyxa brayi</i> 1	EU729697	<i>Cephalopholis boenak</i> (Perciformes: Serranidae)	Gunter and Adlard 2009
<i>Ceratomyxa brayi</i> 2	EU729699	<i>Cephalopholis boenak</i> (Perciformes: Serranidae)	Gunter and Adlard 2009
<i>Ceratomyxa bryanti</i>	EU440357	<i>Abudefduf whitleyi</i> (Perciformes: Pomacentridae)	Gunter and Adlard 2008
<i>Ceratomyxa burgerae</i>	EU440366	<i>Dascyllus aruanus</i> (Perciformes: Pomacentridae)	Gunter and Adlard 2008
<i>Ceratomyxa capricornensis</i>	EU440364	<i>Dischistodus perspicillatus</i> (Perciformes: Pomacentridae)	Gunter and Adlard 2008
<i>Ceratomyxa cheilinae</i>	EU045331	<i>Cheilinus chlorourus</i> (Perciformes: Labridae)	Heiniger et al. 2008
<i>Ceratomyxa choerodona</i>	EU045333	<i>Choerodon cyanodus</i> (Perciformes: Labridae)	Heiniger et al. 2008
<i>Ceratomyxa cribbi</i>	EU440367	<i>Pomacentrus chrysurus</i> (Perciformes: Pomacentridae)	Gunter and Adlard 2008
<i>Ceratomyxa cutmorei</i>	EU729691	<i>Epinephelus fasciatus</i> (Perciformes: Serranidae)	Gunter and Adlard 2009
<i>Ceratomyxa diamanti</i>	FJ204246	<i>Polydactylus macrochir</i> (Perciformes: Polydactylidae)	Gunter et al. 2009
<i>Ceratomyxa dennisi</i>	EU440358	<i>Acanthochromis polyacanthus</i> (Perciformes: Pomacentridae)	Gunter and Adlard 2008
<i>Ceratomyxa ernsti</i>	FJ204247	<i>Sillago ciliata</i> (Perciformes: Sillaginidae)	Gunter et al. 2009
<i>Ceratomyxa falcatus</i>	EU440361	<i>Abudefduf whitleyi</i> (Perciformes: Pomacentridae)	Gunter and Adlard 2008
<i>Ceratomyxa gleasoni</i>	EU729693	<i>Plectropomus leopardus</i> (Perciformes: Serranidae)	Gunter and Adlard 2009
<i>Ceratomyxa hallletae</i>	FJ204248	<i>Lethrinus harak</i> (Perciformes: Lethrinidae)	Gunter et al. 2009
<i>Ceratomyxa heinigeriae</i>	FJ204249	<i>Choerodon cephalotes</i> (Perciformes: Labridae)	Gunter et al. 2009
<i>Ceratomyxa hooperi</i>	EU729692	<i>Epinephelus quoyanus</i> (Perciformes: Serranidae)	Gunter and Adlard 2009
<i>Ceratomyxa jonesi</i>	FJ204250	<i>Pseudolabrus guentheri</i> (Perciformes: Labridae)	Gunter et al. 2009
<i>Ceratomyxa kenti</i>	EU440363	<i>Abudefduf sexfasciatus</i> (Perciformes: Pomacentridae)	Gunter and Adlard 2008
<i>Ceratomyxa koieae</i> sp. n.	GU136392	<i>Sphyaena forsteri</i> (Perciformes: Sphyaenidae)	This paper
<i>Ceratomyxa labracis</i>	AF411472	<i>Dicentrarchus labrax</i> (Perciformes: Moronidae)	Palenzuela et al. 2002
<i>Ceratomyxa lunula</i> 1	EU440376	<i>Neoglyphidon melas</i> (Perciformes: Pomacentridae)	Gunter and Adlard 2008
<i>Ceratomyxa lunula</i> 2	EU440377	<i>Neoglyphidon melas</i> (Perciformes: Pomacentridae)	Gunter and Adlard 2008
<i>Ceratomyxa lunula</i> 3	EU440378	<i>Neoglyphidon melas</i> (Perciformes: Pomacentridae)	Gunter and Adlard 2008
<i>Ceratomyxa milleri</i>	FJ204251	<i>Lutjanus fulviflamma</i> (Perciformes: Lutjanidae)	Gunter et al. 2009
<i>Ceratomyxa moseri</i>	EU440360	<i>Pomacentrus wardi</i> (Perciformes: Pomacentridae)	Gunter and Adlard 2008
<i>Ceratomyxa nolani</i> 1	EU729695	<i>Epinephelus quoyanus</i> (Perciformes: Serranidae)	Gunter and Adlard 2009
<i>Ceratomyxa nolani</i> 2	EU729698	<i>Epinephelus quoyanus</i> (Perciformes: Serranidae)	Gunter and Adlard 2009
<i>Ceratomyxa nowakae</i>	FJ204252	<i>Gymnocranius audleyi</i> (Perciformes: Lethrinidae)	Gunter et al. 2009
<i>Ceratomyxa oxycheilinae</i>	EU045334	<i>Oxycheilinus digramma</i> (Perciformes: Labridae)	Heiniger et al. 2008
<i>Ceratomyxa pantherini</i> sp. n.	GU136393	<i>Bothus pantherinus</i> (Pleuronectiformes: Bothidae)	This paper
<i>Ceratomyxa reidi</i> sp. n.	GU136394	<i>Chaetodon vagabundus</i> (Perciformes: Chaetodontidae)	This paper
<i>Ceratomyxa robertsthomsoni</i>	FJ204253	<i>Liza vaigiensis</i> (Mugiliformes: Mugilidae)	Gunter et al. 2009
<i>Ceratomyxa sewelli</i>	EU440362	<i>Pomacentrus wardi</i> (Perciformes: Pomacentridae)	Gunter and Adlard 2008
<i>Ceratomyxa shasta</i>	AF001579	<i>Oncorhynchus mykiss</i> (Salmoniformes: Salmonidae)	Bartholomew et al. 1997
<i>Ceratomyxa</i> sp.	GU136395	<i>Zebrasoma veliferum</i> (Perciformes: Acanthuridae)	This paper
<i>Ceratomyxa</i> sp. M0304	FJ417043	<i>Gadus morhua</i> (Gadiformes: Gadidae)	Bartošová et al. 2009
<i>Ceratomyxa</i> sp. RM	DQ333429	<i>Siganus rivulatus</i> (Perciformes: Siganidae)	–
<i>Ceratomyxa</i> sp. SAR	DQ333430	<i>Siganus rivulatus</i> (Perciformes: Siganidae)	–
<i>Ceratomyxa</i> sp. SRR	DQ333431	<i>Siganus rivulatus</i> (Perciformes: Siganidae)	–
<i>Ceratomyxa</i> sp. VL	DQ333432	<i>Variola louti</i> (Perciformes: Serranidae)	–
<i>Ceratomyxa</i> sp. 1	DQ377698	<i>Scyliorhinus canicula</i> (Carcharhiniformes: Scyliorhinidae)	Fiala 2006
<i>Ceratomyxa</i> sp. 2	DQ377699	<i>Notacanthus bonaparte</i> (Notacanthiformes: Notacanthidae)	Fiala 2006
<i>Ceratomyxa sparusaurati</i>	AF411471	<i>Sparus aurata</i> (Perciformes: Sparidae)	Palenzuela et al. 2002
<i>Ceratomyxa talboti</i>	EU440368	<i>Dischistodus chrysopoecilus</i> (Perciformes: Pomacentridae)	Gunter and Adlard 2008
<i>Ceratomyxa thalassomae</i>	EU045332	<i>Thalassoma lunare</i> (Perciformes: Labridae)	Heiniger et al. 2008
<i>Ceratomyxa vikrami</i> *	FJ417044	<i>Zeus faber</i> (Zeiformes: Zeidae)	Bartošová et al. 2009
<i>Ceratomyxa whippsi</i>	EU729694	<i>Cephalopholis boenak</i> (Perciformes: Serranidae)	Gunter and Adlard 2009
<i>Ceratomyxa yokoyamai</i>	EU729696	<i>Epinephelus maculatus</i> (Perciformes: Serranidae)	Gunter and Adlard 2009
<i>Ellipsomyxa gobii</i>	AY505126	<i>Pomatoschistus microps</i> (Perciformes: Gobiidae)	Køie et al. 2004
<i>Palliatius indecorus</i>	DQ377712	<i>Alepocephalus grandis</i> (Osmeriformes: Alepocephalidae)	Fiala 2006

* Transferred from *Leptothecca* to *Ceratomyxa* by Gunter and Adlard (2010).

Table 2. Morphometrics of new *Ceratomyxa* species. Mean spore dimensions in μm or $^\circ$ (range in parentheses). L – length; T – thickness; S – sutural position; S% – percentage thickness of smaller valve (S); W – width; PCL – polar capsule length; PCW – polar capsule width; PA – posterior angle (dimension is in $^\circ$).

<i>Ceratomyxa</i> species	Host species	L	T	S	S%	W	PCL	PCW	PA
<i>Ceratomyxa bartholomewae</i> sp. n.	<i>Hyporhamphus dussumieri</i>	5.5 (4.7–6.2)	17.5 (13.5–21.7)	7.6 (5.6–9.9)	43.4 (36.9–50)	5.4 (5.0–5.9)	2.3 (1.8–2.8)	2.3 (1.6–2.7)	168.5 (154.6–183.1)
<i>Ceratomyxa koieae</i> sp. n.	<i>Sphyræna forsteri</i>	5.5 (4.9–6.7)	36.7 (28.5–41.2)	17.5 (13.8–20.0)	47.8 (43.7–49.9)	5.3 (5.1–5.4)	2.8 (2.5–3.2)	2.6 (2.3–2.9)	168.7 (156.3–183.8)
<i>Ceratomyxa pantherini</i> sp. n.	<i>Bothus pantherinus</i>	8.1 (7.3–9.1)	21.6 (17.9–24.6)	10.1 (8.4–11.9)	46.8 (41.5–49.8)	8.0 (7.1–8.9)	2.3 (1.8–2.6)	2.2 (1.7–2.6)	119.1 (85.7–156.7)
<i>Ceratomyxa reidi</i> sp. n.	<i>Chaetodon vagabundus</i>	6.8 (5.8–7.5)	17.5 (14.3–20.7)	7.9 (6.5–9.2)	45.1 (39.8–48.6)	6.5 (6.0–7.0)	2.1 (1.7–2.4)	2.0 (1.7–2.5)	141.2 (102.9–170.3)

Table 3. Morphometric comparison of *Ceratomyxa* species from bothid fishes. Bold measurements do not overlap with those of *C. pantherini*. Bold italicised measurement ranges overlap partially with those of *C. pantherini* but means are distinct. Normal text measurements are superficially similar to *C. pantherini*. For abbreviations see Table 2.

<i>Ceratomyxa</i> species	L	T	W	PCL	PCW	PAA
<i>C. pantherini</i> sp. n.	8.1 (7.3–9.1)	21.60 (17.9–24.6)	8.0 (7.1–8.9)	2.3 (1.8–2.6)	2.3 (1.7–2.6)	119 (86–157)
<i>C. faba</i> Meglitsch, 1960	6.2 (5.6–6.7)	12.7 (10.7–14.1)	6.4 (6.1–7.0)	2.4 (2.0–3.1)	2.4 (2.0–3.1)	162 (125–180)
<i>C. hama</i> Meglitsch, 1960	7.4 (6.8–8.3)	23.4 (18.5–29.8)	7.7 (7.0–8.3)	3.2 (2.0–3.5)	3.2 (2.0–3.5)	111 (89–152)
<i>C. laxa</i> Meglitsch, 1960	8.7 (7.8–9.8)	25.6 (20.8–30.3)	–	3.4 (2.9–3.9)	3.4 (2.9–3.9)	130 (93–173)
<i>C. renalis</i> * Meglitsch, 1960	8.0 (7.3–8.5)	27.7 (22.5–33.4)	8.9 (7.7–9.3)	3.1 (2.7–3.2)	3.1 (2.7–3.2)	118 (90–134)
<i>C. torquata</i> Meglitsch, 1960	8.1 (7.8–9.5)	26.9 (20.5–30.3)	9.2 (8.3–10.1)	3.8 (2.8–4.7)	3.8 (2.8–4.7)	135 (115–178)
<i>C. vepallida</i> Meglitsch, 1960	8.6 (7.8–9.6)	18.7 (16.0–21.4)	8.1	3.2 (2.9–3.6)	2.9 (2.5–3.2)	158 (142–180)

* *C. renalis* infects the urinary bladder.

in the collections of the Queensland Museum, Brisbane, Australia.

E t y m o l o g y : In honour of Marianne Køie, University of Copenhagen, Denmark, for her research into marine myxosporean life cycles.

Remarks: *Ceratomyxa koieae* is superficially similar to *Ceratomyxa epinepheli* Wu, Wu et Dingke, 1993, *C. huanghaiensis* Zhao et Song, 2003 and *C. urophysis* Fantham, Porter et Richardson, 1940. *Ceratomyxa epinepheli* has shorter spores with smaller polar capsules ($5.3; 2.6 \times 2.6 \mu\text{m}$) than *C. koieae* ($5.5; 2.8 \times 2.8 \mu\text{m}$). *Ceratomyxa koieae* has shorter spores ($5.5 \mu\text{m}$) than *C. huanghaiensis* ($6.5 \mu\text{m}$). Also, the valves of *C. huanghaiensis* are unequal. The description of *C. urophysis* only contains ranges for spore length and thickness ($5\text{--}7.5 \times 25\text{--}39.1 \mu\text{m}$) and both these measurements overlap with those of *C. koieae* ($4.9\text{--}6.7 \times 28.5\text{--}41 \mu\text{m}$) but the figure depicts *C. urophysis* with pyriform polar capsules which are distinct from the subspherical polar capsules of *C. koieae*.

A total of 1,379 bases of SSU rDNA was generated for *Ceratomyxa koieae* (GenBank accession GU136392). The sequence differs from the aligned sequences of *Ceratomyxa* spp. at 148–299 of the 1,664 nucleotide alignment and has a maximum genetic similarity of 87.7% with *C. jonesi*. *Ceratomyxa koieae* is the first described species of this genus recorded from a species of Sphyrænidae (Perciformes).

Ceratomyxa pantherini sp. n.

Figs. 2G–I, 4A–D, Table 2

Spores (Figs. 2G–I, 4A–D): Mature spore slightly crescent-shaped in lateral view, 8.1 ± 0.4 (7.3–9.1) in length and 21.6 ± 2.0 (17.9–24.6) in thickness ($n = 30$). Cylindrical or rarely sigmoid in apical view, 8.0 ± 0.5 (7.1–8.9) in width ($n = 9$). Posterior angle strongly to moderately concave ($85.7\text{--}156.7^\circ$). Valves almost equal in size, smoothly rounded in lateral view. Straight suture visible between valves. Subspherical polar capsules, 2.3 ± 0.2 (1.8–2.6) in length and 2.2 ± 0.2 (1.7–2.6) in width ($n = 30$), positioned at apex of spore. In apical view the polar capsules are not located centrally but are positioned touching one wall of the valves (see Fig. 4B).

Plasmodia: Disporous and polysporous (Fig. 4 C, D).

Type host: *Bothus pantherinus* (Rüppell) (leopard flounder), Family Bothidae.

Type locality: Off Lizard Island, Great Barrier Reef, Queensland ($14^\circ 39'S$, $145^\circ 27'E$).

Site of infection: Gall bladder.

Prevalence: 1 of 1 (100%).

Type material: Syntypes – air-dried slides stained with Giemsa, registration numbers G465410–465412, deposited in the collections of the Queensland Museum, Brisbane, Australia.

E t y m o l o g y : Specific name refers to the host species.

Fig. 4. *Ceratomyxa pantherini* sp. n. from *Bothus pantherinus*; photomicrographs of spores. **A** – sutural view; **B** – apical view, note polar capsules are not central; **C** – polysporous plasmodium; **D** – disporous plasmodium. Scale bar = 10 μ m.

Remarks: *Ceratomyxa pantherini* is the first species described in the genus with polar capsules not located centrally in apical view. It is superficially similar to *Ceratomyxa artemiellii* Polyanskii, 1955, *C. blennius* Noble, 1938, *C. diloba* Dogiel, 1948, *C. hama* Meglitsch, 1960, *C. lagocephali* Kpatcha, Diebakate, Faye et Toguebaye, 1996 and *C. laxa* Meglitsch, 1960. It can be distinguished from all these species on the basis of the shape and size of the polar capsules. The polar capsules of *C. pantherini* are smaller ($2.3 \times 2.2 \mu\text{m}$) than those of *C. artemiellii* ($3.6 \times 3.6 \mu\text{m}$), *C. blennius* ($3 \times 3 \mu\text{m}$), *C. diloba* ($3 \times 3 \mu\text{m}$), *C. hama* ($3.2 \times 3.5 \mu\text{m}$), *C. lagocephali* ($4.2 \times 4.5 \mu\text{m}$) and *C. laxa* ($3.4 \times 3.4 \mu\text{m}$).

A total of 1,489 bases of SSU rDNA was generated for *Ceratomyxa pantherini* (GenBank accession GU136393). The sequence differs from the aligned sequences of *Ceratomyxa* spp. at 206–367 of the 1,664 nucleotide alignment and has a maximum genetic similarity of 83.6% with *C. anko*.

Only one other species of bothid is known to harbour a species of *Ceratomyxa*. *Arnoglossus scapha* (= *Caulopsetta scapha*) is host to seven species: *C. faba*, *C. hama*, *C. laxa*, *C. renalis*, *C. torquata*, *C. uncinata*, and *C. vellapida* (see Meglitsch 1960), the richest fauna of *Ceratomyxa* known from a single host. Although all species overlap slightly, each can be distinguished from *C. pantherini* (Table 3).

***Ceratomyxa reidi* sp. n.** Figs. 2J–L, 5A, B, Table 2

Spores (Figs. 2J–L, 5A, B): Mature spore slightly crescent-shaped in lateral view, 6.8 ± 0.4 (5.8–7.5) in length and 17.5 ± 1.5 (14.3–20.7) in thickness ($n = 30$). Cylindrical in apical view, 6.5 ± 0.4 (6.0–7.0) in width ($n = 7$). Posterior angle strongly to slightly concave (102.9 – 170.3°). Valves slightly unequal in size, smoothly ovoid in lateral view. Straight suture visible between valves. Subspherical polar capsules, 2.1 ± 0.2 (1.7–2.4) in length and 2.0 ± 0.2 (1.7–2.5) in width ($n = 30$), positioned at apex of spore, centrally located in apical view.

Plasmodia: Monosporous.

Type host: *Chaetodon vagabundus* Linnaeus (Indian vagabond butterflyfish), Family Chaetodontidae.

Fig. 5. *Ceratomyxa reidi* sp. n. from *Chaetodon vagabundus*; photomicrographs of spores. **A** – sutural view; **B** – apical view. Scale bar = 10 μ m.

Type locality: Off Lizard Island, Great Barrier Reef, Queensland ($14^\circ 39'S$, $145^\circ 27'E$).

Site of infection: Gall bladder.

Prevalence: 5 of 22 (22.7%).

Type material: Syntypes – air-dried slides stained with Giemsa, registration numbers G465413–465415, deposited in the collections of the Queensland Museum, Brisbane, Australia.

Etymology: In honour of Anthony Reid, The University of Queensland.

Remarks: *Ceratomyxa reidi* is superficially similar to *Ceratomyxa cottoidi* Reed, Basson, Van As et Dyková, 2007, *C. cutmorei* Gunter et Adlard, 2009, *C. gibba* and *C. lateolabracis* Zhao et Song, 2003. *Ceratomyxa reidi* has thicker spores and smaller polar capsules (17.5 ; $2.1 \times 2.0 \mu\text{m}$) than *C. cutmorei* (16.1 ; $2.4 \times 2.3 \mu\text{m}$) and *C. gibba* (17 ; $2.8 \times 2.3 \mu\text{m}$). *Ceratomyxa cottoidi* can be distinguished by having thicker spores and larger polar capsules (18.2 ; $2.7 \times 2.4 \mu\text{m}$) than *C. reidi* (17.5 ; $2.1 \times 2.0 \mu\text{m}$). The spores of *C. lateolabracis* are thinner ($16.7 \mu\text{m}$) than *C. reidi* ($17.5 \mu\text{m}$) and can also be distinguished by being more ovoid compared to the spores of *C. reidi*, which taper to a rounded point.

A total of 1,493 bases of SSU rDNA was generated for *Ceratomyxa reidi* (GenBank accession GU136394) and was obtained from a single infection only. The sequence differs from the aligned sequences of *Ceratomyxa* spp. at 145–346 of the 1,664 nucleotide alignment and has a maximum genetic similarity of 88.7% with the un-

Fig. 6. *Ceratomyxa* sp. from *Zebrasoma veliferum*; photomicrographs of spores. **A** – sutural view; **B** – immature spore apical view; **C** – disporous plasmodium; **D** – monosporous plasmodium. Scale bar = 10 μ m.

described species of *Ceratomyxa* ex *Zebrasoma veliferum* reported in this study.

Only one other *Ceratomyxa* species has been described from chaetodontids. *Ceratomyxa sprengi* Moser, Kent et Dennis, 1989 was described from *Chaetodon aureofasciatus* and *C. rainfordi*, but also from fishes of other families: *Lutjanus amabilis* (Lutjanidae), *Platycephalus bosschei* (Platycephalidae) and *Choerodon venustus* (Labridae) off Heron Island, Australia. Although, both species are found in chaetodontids collected from the Great Barrier Reef they are morphologically distinct. On average, the spores of *C. reidi* are longer, thicker and wider ($6.8 \times 17.5 \times 7.9 \mu\text{m}$) than *C. sprengi* ($5.7 \times 16.3 \times 3.5\text{--}5 \mu\text{m}$) and the polar capsules of *C. reidi* are slightly smaller ($2.1 \times 2.0 \mu\text{m}$) than those of *C. sprengi* ($2.4 \times 2.4 \mu\text{m}$). On the basis of morphometric and host data one can conclude that these species are distinct.

Ceratomyxa sp. ex *Zebrasoma veliferum*

Spores and plasmodia of *Ceratomyxa* sp. were found in the gall bladder of 1 of 1 (100%) individual of *Zebrasoma veliferum* (Bloch) (sailfin tang), Family Acanthuridae, collected off Lizard Island, Great Barrier Reef, Queensland ($14^{\circ}39'S$, $145^{\circ}27'E$). Unfortunately, since infection mainly consisted of immature spores in plasmodia (Fig. 6B–D), insufficient material (mature spores) was available for a thorough morphometric characterisation; thus, we prefer not to describe it as a new species at this time. Only three mature spores free of plasmodia were observed. These spores were elongate ovoid in lateral view, 6.2 ($5.9\text{--}6.8$) μm in length and 16.8 ($15.9\text{--}17.6$) μm in thickness ($n = 3$) (Figs. 2M, N, 6A). Valves slightly unequal in size, smoothly ovoid in lateral view. Straight suture visible between valves. Subspherical polar capsules, 2.4 ($2.3\text{--}2.5$) μm in length and 2.4 ($2.3\text{--}2.5$) μm in width ($n = 3$), positioned at apex of spore, centrally located in apical view (Fig. 6B).

The plasmodia were disporous and monosporous (Fig. 6C, D). A total of 1,426 bases of SSU rDNA was generated for *Ceratomyxa* sp. ex *Zebrasoma veliferum* (GenBank accession GU136395). The sequence differs from the aligned sequences of *Ceratomyxa* spp. at 145–

316 of the 1,664 nucleotide alignment and has a maximum genetic similarity of 88.7% with *Ceratomyxa reidi*.

Material: Voucher specimens – air-dried spores on slides stained with Giemsa, registration numbers G465416–465417, deposited in the collections of the Queensland Museum, Brisbane, Australia.

Remarks: Only one other species of *Ceratomyxa* has been described from an acanthurid. *Ceratomyxa acanthuri* Kpatcha, Diebakate, Faye et Toguebaye, 1996 was described from the gall bladder of *Acanthurus monroviae* off the Senegal coast. The spores of *C. acanthuri* are superficially similar to the *Ceratomyxa* sp. ex *Zebrasoma veliferum*. Although the thickness of the spores are similar ($16.5 \mu\text{m}$ *C. acanthuri*, 16.8 *Ceratomyxa* sp. ex *Zebrasoma veliferum*), the spores of *C. acanthuri* are longer ($10.5 \mu\text{m}$) and the polar capsules are also slightly larger ($2.75 \times 2.75 \mu\text{m}$) than *Ceratomyxa* sp. ex *Zebrasoma veliferum* ($6.2 \mu\text{m}$ and $2.4 \times 2.4 \mu\text{m}$). On the basis of host and slight morphometric differences we believe the species from *Zebrasoma veliferum* is not *C. acanthuri* but represents a distinct and novel species.

Molecular phylogeny

Sequences from 50 *Ceratomyxa* species were included in the molecular analyses. Neighbour joining, parsimony, maximum likelihood and Bayesian analyses produced trees with similar topology but had varying levels of clade support (see Fig. 7). The phylogenetic position of *Ceratomyxa* species and resolution of clades was near identical to that reported in the recent phylogeny of *Ceratomyxa* (Gunter et al. 2009). Few relationships between species are strongly supported in all analyses because alternate relationships with other species are just as likely.

DISCUSSION

Since the incorporation of molecular systematics into myxosporean taxonomy, SSU rDNA data have shown that morphologically similar species are not necessarily closely related and other biological characteristics may be important descriptive characters that correlate with genetic

Fig. 7. Phylogenetic tree resulting from Bayesian analysis inferred from the SSU rDNA data-set. Support values at branching points are indicated in shades from black (100) to white (<50) and symbols representing intervals of 10 in the nodal boxes as: clade credibility values (CCV) from Bayesian inference (BI: top left); bootstrap values from maximum likelihood (ML: bottom left); bootstrap values from parsimony analyses (P: top right) and bootstrap values from neighbour joining analyses (NJ: bottom right).

relatedness (Whipps et al. 2004, Fiala 2006, Gunter et al. 2009). Tissue tropism is an informative characteristic for discrimination of species in the genera *Myxobolus*, *Heneguya* and *Kudoa* (see Burger et al. 2006, Fiala 2006), but is uninformative for genera that primarily infect a single tissue, i.e. *Ceratomyxa* and *Coccomyxa*, species of which are typically restricted to the gall bladder. Molecular studies have revealed that *Myxobolus* and *Ceratomyxa* species tend to be highly host specific (Gunter et al. 2009, Molnár et al. 2009) and as such host identity may represent an important diagnostic character. The *Ceratomyxa* species characterised here are from distantly related families of fishes, each *Ceratomyxa* species is genetically distinct and appears to be restricted to a single host species. These results support the hypothesis that *Ceratomyxa* are generally highly host specific. It is becoming more apparent that host species is an informative character for species discrimination in ceratomyxids. Ideally, modern taxonomy should incorporate molecular characterisation, however, not all workers in the field have this capacity. The utility of host as an informative diagnostic character is particularly valuable as species discrimination based on spore morphology alone lacks a level of confidence (particularly in species-rich genera) due to a relative paucity of morphometric characters together with small spore size and morphological plasticity.

The five species characterised here using 18S rRNA form close relationships with other sequenced *Ceratomyxa* species (see Fig. 7). The position of *C. bartholomewae* remains unresolved possibly due to it being the only parasite of beloniformes in the analysis. *Ceratomyxa reidi* and *Ceratomyxa* sp. ex *Zebrasoma veliferum* form a supported clade with *C. atkinsoni*, a species from a lethriniid also from Lizard Island. *Ceratomyxa koieae* sits basally in a clade of pomacentrid-infecting *Ceratomyxa* spp. While *C. pantherini* forms a clade with *C. anko*, *C. vikrami*, *C. cheilinae* and *C. oxycheilinae*, but only the relationship with *C. anko* is supported in all analyses. Two spe-

cies described here share taxonomic affinities with other *Ceratomyxa* species included in the molecular analyses. Superficially, *C. bartholomewae* and *C. robertsthomsoni* are similar, as are *C. reidi* and *C. cutmorei*. However in both cases, neither pair of morphologically similar species is more closely related to each other than to other *Ceratomyxa* in the analyses.

The molecular analyses contain *Ceratomyxa* species from a diverse range of fishes including representatives from 12 orders. The vast majority of species are from perciform fishes but the dataset includes a single species from a beloniform, a carcharhiniiform, a clupeiform, a gadiform, a lophiiform, a mugiliform, a notacanthiform, an osmeriform, a pleuronectiform, a salmoniform and a zeiform. Interestingly, the position of some *Ceratomyxa* species from non-perciform fishes remains relatively unresolved (e.g. *C. auerbachii* from a clupeiform, *C. bartholomewae* from a beloniform, *Ceratomyxa* sp. M0304 from a gadiform, *C. vikrami* from a zeiform). *Ceratomyxa pantherini* and *C. anko* always form a strong relationship and in Bayesian analysis and maximum likelihood also form a clade with *C. vikrami*; these species infect a pleuronectiform, a lophiiform and a zeiform, respectively. These relationships raise questions about the evolution and speciation of *Ceratomyxa*. Further research is needed to investigate whether radiation associated with radiation among fish orders is occurring and the inclusion of more species from a diverse range of hosts may also improve the resolution of these clades.

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REFERENCES

- BARTHOLOMEW J.L., WHIPPLE M.J., FRYER J.L. 1997: The life cycle of *Ceratomyxa shasta*, a myxosporean parasite of salmonids, requires a freshwater polychaete as an alternate host. *J. Parasitol.* 83: 859–869.
- BARTOŠOVÁ P., FIALA I., HYPŠA V. 2009: Concatenated SSU and LSU rDNA data confirm the main evolutionary trends within myxosporeans (Myxozoa: Myxosporea) and provide an effective tool for their molecular phylogenetics. *Mol. Phylogenet. Evol.* 53: 81–93.
- BURGER M.A.A., CRIBB T.H., ADLARD R.D. 2007: Patterns of relatedness in the Kudoidea with descriptions of *Kudoa chaetodoni* sp. n. and *K. lethrini* sp. n. (Myxosporea: Multivalvulida). *Parasitology* 134: 669–681.
- FIALA I. 2006: The phylogeny of the Myxosporea (Myxozoa) based on small subunit ribosomal RNA gene analysis. *Int. J. Parasitol.* 36: 1521–1534.
- FREEMAN M.A., YOKOYAMA H., OGAWA K. 2008: Description and phylogeny of *Ceratomyxa anko* sp. n. and *Zschokkella lophii* sp. n. from the Japanese anglerfish, *Lophius litulon* (Jordan). *J. Fish Dis.* 31: 921–930.
- GUNTER N.L., ADLARD R.D. 2008: Bivalvulidan (Myxozoa: Myxosporea) parasites of damselfishes with description of twelve novel species from Australia's Great Barrier Reef. *Parasitology* 135: 1165–1178.
- GUNTER N.L., ADLARD R.D. 2009: Seven new species of *Ceratomyxa* from the gall bladders of serranids from the Great Barrier Reef, Australia. *Syst. Parasitol.* 73: 1–11.
- GUNTER N., ADLARD R. 2010: The demise of the genus *Leptotheca* Thélohan, 1895 (Myxozoa: Myxosporea: Ceratomyxidae) and assignation of the species to *Ceratomyxa* Thélohan, 1892 (Myxosporea: Ceratomyxidae), *Ellipsomyxa* Koie, 2003 (Myxosporea: Ceratomyxidae), *Myxobolus* Bütschli, 1882 and

- Sphaerospora* Thélohan, 1892 (Myxosporea: Sphaerosporidae). Syst. Parasitol. 75: 81–104.
- GUNTER N.L., WHIPPS C.M., ADLARD R.D. 2009: *Ceratomyxa* (Myxozoa: Bivalvulida): robust taxon or genus of convenience? Int. J. Parasitol. 39: 1395–1405.
- HALL T.A. 1999: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41: 95–98.
- HEINIGER H., GUNTER N.L., ADLARD R.D. 2008: Relationships between four novel ceratomyxid parasites from the gall bladders of labrid fishes from Heron Island, Australia. Parasitol. Int. 57: 158–165.
- KØIE M., KARLSBAKK E., NYLUND A. 2008: The marine herring myxozoan *Ceratomyxa auerbachii* (Myxozoa: Ceratomyxidae) uses *Chone infundibuliformis* (Annelida: Polychaeta: Sabelliidae) as invertebrate host. Folia Parasitol. 55: 100–104.
- KØIE M., WHIPPS C.M., KENT M.L. 2004: *Ellipsomyxa gobicola* (Myxozoa: Ceratomyxidae) in the common goby *Pomatoschistus microps* (Teleostei: Gobiidae) uses *Nereis* spp. (Annelida: Polychaeta) as invertebrate hosts. Folia Parasitol. 51: 14–18.
- LOM J., ARTHUR J.R. 1989: A guideline for the preparation of species descriptions in Myxosporea. J. Fish Dis. 12: 151–156.
- MEGLITSCH P.A. 1960: Some coelozoic myxosporidia from New Zealand fishes. I. General and family Ceratomyxidae. Trans. R. Soc. N.Z. 88: 265–365.
- MOLNÁR K., SZÉKELY C., HALLETT S., ATKINSON S. 2009: Some remarks on the occurrence, host-specificity and validity of *Myxobolus rotundus* Nemeček, 1911 (Myxozoa: Myxosporea) Syst. Parasitol. 72: 71–79.
- MOSER M., KENT M.L., DENNIS D. 1989: Gall bladder Myxosporea in coral reef fishes from Heron Island, Australia. Aust. J. Zool. 37: 1–13.
- PALENZUELA O., REDONDO M.J., ALVAREZ-PELLITERO P. 2002: Description of *Enteromyxum scopthalmi* gen. nov., sp. nov. (Myxozoa), an intestinal parasite of turbot (*Scophthalmus maximus* L.) using morphological and ribosomal RNA sequence data. Parasitology 124: 369–379.
- POSADA D., CRANDALL K.A. 1998: Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817–818.
- RONQUIST F., HUELSENBECK J.P. 2003: MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574.
- SWOFFORD D.L. 2003: PAUP*. Phylogenetic analysis using parsimony (*and other methods). Massachusetts, Sinauer Associates, Sunderland.
- THOMPSON J.D., HIGGINS D.G., GIBSON T.J. 1994: Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673–4680.
- WHIPPS C.M., ADLARD R.D., BRYANT M.S., LESTER R.J.G., FINDLAY V., KENT M.L. 2003: First report of three *Kudoa* species from Eastern Australia: *Kudoa thyrsites* from Mahi mahi (*Coryphaena hippurus*), *Kudoa amamiensis* and *Kudoa minithyrsites* sp. n. from Sweeper (*Pempheris ypsilychnus*). J. Eukaryot. Microbiol. 50: 215–219.
- WHIPPS C.M., GROSSEL G., ADLARD R.D., YOKOYAMA H., BRYANT M.S., MUNDAY B.L., KENT M.L. 2004: Phylogeny of the Multivalvulidae (Myxozoa: Myxosporea) based on comparative ribosomal DNA sequence analysis. J. Parasitol. 90: 618–622.

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