DOI: 10.2478/s11686-013-0149-3

Acta Parasitologica, 2013, 58(3), 342–360; ISSN 1230-2821

VERSITA

Molecular identification of cryptic species of *Ceratomyxa* Thélohan, 1892 (Myxosporea: Bivalvulida) including the description of eight novel species from apogonid fishes (Perciformes: Apogonidae) from Australian waters

Holly Heiniger[1, 2] and Robert D. Adlard[1, 2]*

[1]Natural Environments Program, Queensland Museum, PO Box 3300, South Brisbane, Queensland 4101, Australia;
[2]School of Biological Sciences, The University of Queensland, Brisbane, Queensland 4072, Australia

Abstract

Ceratomyxa parasites from the gall bladders of 23 species of cardinalfishes (family Apogonidae) from Australian waters were examined for their taxonomic identity and phylogenetic relatedness. We identified 15 of the 23 apogonid fish species infected with species of *Ceratomyxa*. Although the majority of apogonid species harboured only a single *Ceratomyxa* species, four were found with multiple species of *Ceratomyxa*. This study describes eight novel species using a combination of morphological, small subunit ribosomal DNA (SSU rDNA) and biological characters. Six *Ceratomyxa* species are reported from single apogonid species, while two are reported from multiple host species. Molecular data were critical in identifying several morphologically cryptic species. However, our results suggest that SSU rDNA was not capable of distinguishing all the species present in the current study system and alternative genetic markers should be investigated in the future.

Keywords

Ceratomyxa, Myxosporea, Bivalvulida, Apogonidae, cryptic species, host specificity, phylogeny, diversity, parasite

Introduction

The teleost family Apogonidae (Cardinalfishes) forms a major component of the coral reef fish assemblage, both from their species richness and numerical abundance (Allen 1993). The majority of species are marine, inshore reef inhabitants, feeding nocturnally on benthic and pelagic plankton, invertebrates and small fish (Kuiter and Kozawa 1999). Day resting sites include either caves and crevices (mostly solitary or small groups of a single species) or around and above branching corals (often large multi-species aggregations) (Greenfield and Johnson 1990). To date, only a single species of the myxosporean genus *Ceratomyxa* Thélohan, 1892 has been described from an apogonid. *Ceratomyxa apogoni* (described as *Leptotheca apogoni*) (Narasimhamurti, Kalavati, Anuradha and Padma Dorothy, 1990) (Gunter and Adlard, 2010) has been recorded from *Ostorhinchus aureus* (as *Apogon aureus*) from the Bay of Bengal, India (Narasimhamurti *et al*. 1990).

Ceratomyxa is the second richest myxozoan genus, containing over 270 species described to date (Gunter and Adlard 2010). Species of this bivalvulidan genus are mainly coelozoic, and found often in marine teleost fishes and rarely in freshwater fishes (Gunter and Adlard 2010; Lom and Dykova 2006). To date, 42 species have been described from the gall bladders of fishes collected from Australian waters (of which 39 are described from the Great Barrier Reef off Queensland) (Moser *et al*. 1989; Gunter and Adlard 2008, 2009; Gunter *et al*. 2009, 2010; Heiniger *et al*. 2008; Gleeson and Adlard 2011). The inclusion of molecular characters in current myxosporean taxonomy in combination with comprehensive sampling of several host families (Labridae, Pomacentridae, Serranidae and Sparidae) has made it possible to assess the host specificity of many species of *Ceratomyxa* (see Gunter and Adlard 2008, 2009;

*Corresponding author: robert.adlard@qm.qld.gov.au

Heiniger *et al*. 2008; Alama-Bermejo *et al*. 2011). It is now apparent that marine *Ceratomyxa* are, in general, highly host specific, and typically restricted to a single host species. This paper describes eight novel species of *Ceratomyxa* infecting the gall bladders of 15 species of apogonids from Australian waters. The host specificity and species richness of *Ceratomyxa* within the Apogonidae is also assessed.

Materials and Methods

Host and parasite collection

Apogonids were collected using localised sprays of clove oil anaesthetic, spear and micro spear off Heron Island (23°26′S, 151°54′E) and Lizard Island (14°40′S, 145°27′E), Great Barrier Reef, Queensland, and off Point Cloates (22°40′S, 113°41′E), Ningaloo Reef, Western Australia. Fish were euthanized by neural pithing. Apogonid identification was performed with reference to Kuiter (1999) and Fishbase (www.fishbase.org). Currently valid host names were taken from the California Academy of Sciences Catalogue of Fishes (http://research.calacademy.org/ichthyology/catalog/fishcatmain.asp). The gall bladder of each fish was examined for the presence of *Ceratomyxa* infections using the methods described by Heiniger *et al.* (2008). Infected gall bladders were preserved in 100% ethanol for DNA analysis and frozen in saline for morphological characterisation.

Morphological analysis of spores

Measurements of spores followed the guidelines of Lom and Arthur (1989) and also followed further recommendations by Heiniger *et al.* (2008). Images of thirty spores were taken with an Olympus BH2 microscope at 400x or 1000x magnification using a Nikon Digital Sight DS-LI digital camera (Nikon Corporation, Japan). Measurements were taken from microphotographs using the measuring tool in the Nikon NIS Elements software (Nikon Corporation, Japan) calibrated against a stage micrometer. Mean measurements and their standard deviation were calculated for each spore dimension, allowing characterisation of each isolate. All measurements are given in micrometres (μm). Type specimens were deposited in the Queensland Museum (QM) and in the Western Australian Museum (WAM), depending on the origin of material.

Morphological comparisons referred to in the Results section under 'Morphological Affinities' for each species were undertaken to discriminate those species which most closely resembled the new species being described. These species were selected from a dataset of morphometrics that included all *Ceratomyxa* spp. from marine fish. Principal component analyses (PCA) were performed on all new morphometric data using the software PAST version 2.10 (Hammer *et al*. 2001). Scatterplots with 95% confidence ellipses were generated using variant-covariant matrices.

rDNA analysis

DNA was extracted from 600 μl of infected bile preserved in ethanol. The sample was first pelleted at 15 700 g for 10 minutes and the ethanol supernatant removed. DNA was extracted from the pellet as per the recommended protocol accompanying the QIAgen DNeasy Kit (QIAGEN Inc., Valencia, California). Small subunit ribosomal DNA (SSU rDNA) was amplified by PCR using the primers MyxospecF 5' TTC TGC CGT ATC AAC TWG TTG (Fiala 2006) and 18R 5' CTA CGG AAA CCT TGT TAC G (Whipps *et al*. 2003). Large subunit ribosomal DNA (LSU rDNA) was amplified by PCR using the primers NLF184 5' ACCCGCTGAAYTTAAGCATAT (Bartošová *et al*. 2009) and NLR1270 5**'** TTCATCCCGCATCGCCAGTTC (Bartošová *et al*. 2009). PCR reactions and purification were as described by Heiniger *et al*. (2008), with the following exceptions: annealing temperature of 56°C and 25 cycles for LSU rDNA reactions, and extension and final extension temperatures were performed at 72°C in all PCR reactions. Purified DNA was sent to Australian Genome Research Facility, The University of Queensland, Australia, for sequence determination using the same primers as used for the initial amplification.

Phylogenetic analysis

rDNA sequences from the taxa described in this study were edited using Geneious Pro version 5.6.2 (Drummond *et al.* 2010). All sequences generated in this study were lodged in GenBank. Initial phylogenetic analyses of SSU rDNA included 68 *Ceratomyxa* sequences, all with corresponding formal descriptions, for comparative purposes. This dataset was aligned using Muscle version 3.7 (Edgar 2004) using the Clustal W algorithm (Thompson *et al*. 1994) with UPGMB parameters for all interations on the CIPRES portal (Miller *et al*. 2010). Maximum likelihood analysis was conducted using the RAxML algorithm (Stamatakis *et al*. 2008) on the CIPRES portal with the gamma rate model of heterogeneity and maximum likelihood search estimating the proportion of invariable sites parameters. Nodal support was inferred based on 100 bootstrap replicates. This analysis indicated that the new *Ceratomyxa* species reported here fell within the 'marine teleost clade' (Gunter *et al*. 2009; Fiala 2006). Therefore, for brevity, only the new species and closest related *Ceratomyxa* SSU rDNA sequences, as determined by BLAST analyses and Maximum Likelihood analysis described above, were used in phylogenetic analyses presented here. *Ellipsomyxa gobii* Køie, 2003 was used as outgroup taxon in all SSU rDNA analyses. All available LSU rDNA sequences of *Ceratomyxa* and closest related myxosporean species, as determined by BLAST analyses, were used in phylogenetic analyses of the LSU rDNA region. *Unicapsula* sp. (Whipps *et al*. 2004) and *Unicapsula pflugfelderi* Schubert, Sprague et Reinboth, 1975 were used as outgroup taxa in all LSU rDNA analyses. Alignments of SSU and LSU rDNA datasets were produced as described above. The resulting alignments were ex-

Table I. Sample numbers and *Ceratomyxa* infections for 23 apogonid species collected in Australian waters between 2005 and 2012. The numbers of infected fish are listed as a fraction of the total number of fish sampled. Corresponding *Ceratomyxa* species described in this publication are listed for each apogonid host species. Confirmed identities of *Ceratomyxa* infections refer to those identified by DNA sequencing. HI: Heron Island, LI: Lizard Island, NR: Ningaloo Reef

Species	Heron Is.	Lizard Is.	Ningaloo Rf.	Total	*Ceratomyxa* spp.
Apogon doederleini	12/14	1/2	0	13/16	*C. gunterae* sp. nov. 3 confirmed HI *C. cardinalis* sp. nov. 1 confirmed HI, 1 confirmed LI
Archamia bleekeri	0	1	0	1	
Archamia fucata	15/20	13/26	0	28/46	*C. heronensis* sp. nov. 3 confirmed HI *C. archamiae* sp. nov. 2 confirmed HI *C. ireneae* sp. nov. 2 confirmed LI
Archamia zosterophora	0	2/2	0	2/2	*C. ireneae* sp. nov. 1 confirmed
Cercamia eremia	0	1	0	1	
Cheilodipterus artus	0	9/13	0	9/13	*C. cardinalis* sp. nov. 2 confirmed
Cheilodipterus intermedius	4/5	1/19	1	5/25	*C. cardinalis* sp. nov. 3 confirmed HI, 1 confirmed LI
Cheilodipterus macrodon	3	0	0	3	
Cheilodipterus quinquelineatus	12/28	16/34	0	28/62	*C. cardinalis* sp. nov. 3 confirmed HI, 4 confirmed LI
Nectamia fusca	10/32	20/21	0	30/53	*C. cardinalis* sp. nov. 4 confirmed HI, 3 confirmed LI
Nectamia savayensis	0	0	1	1	
Ostorhinchus aureus	0	0	5/14	5/14	*C. ostorhinchi* sp. nov. 1 confirmed *C. cardinalis* sp. nov. 1 confirmed
Ostorhinchus compressus	0	0	5	5	
Ostorhinchus cookii	19/35	4/8	2	23/45	*C. cardinalis* sp. nov. 2 confirmed HI, 1 confirmed LI
Ostorhinchus cyanosoma	0	3/4	11/23	14/27	*C. cyanosomae* sp. nov. 1 confirmed LI *C. cardinalis* sp. nov.1 confirmed LI, 4 confirmed NR
Ostorhinchus novemfasciatus	0	1	0	1	
Ostorhinchus properuptus	0	2/9	0	2/9	*C. cardinalis* sp. nov. 1 confirmed
Ostorhinchus rubrimacula	0	24/29	0	24/29	*C. cardinalis* sp. nov. 1 confirmed
Ostorhinchus rueppellii	0	0	6/16	6/16	*C. rueppellii* sp. nov. 1 confirmed
Pristiapogon exostigma	0	2	0	2	
Rhabdamia gracilis	0	10	0	10	
Zoramia leptacantha	0	37/269	0	37/269	*C. cardinalis* sp. nov. 2 confirmed
Zoramia viridiventer	0	8/74	0	8/74	*C. ireneae* sp. nov. 2 confirmed
Total per locality	**72/137**	**140/525**	**22/62**	**234/724**	

ported as fasta and nexus files, edited by eye and trimmed using MacClade version 4.08 (Maddison and Maddison 2005). This produced alignments of 1464 and 1342 bases for SSU and LSU rDNA, respectively. These alignments were used to conduct all phylogenetic analyses.

Maximum likelihood analyses were performed as described above. Bayesian analyses were conducted using MrBayes version 3.1.2 (Ronquist and Huelsenbeck 2003). The software jModelTest version 0.1.1 (Posada 2008) was used to estimate the best substitution model for the SSU and LSU rDNA datasets. Bayesian inference analysis was conducted using the TIM3+I+G model for SSU and GTR+G model for LSU rDNA datasets, predicted as the best estimator by the Akaike Information Criterion (AIC) in jModelTest. Bayesian inference analyses were run over 10 000 000 generations (ngen = 10 000 000) with 2 runs each containing 4 simultaneous Markov Chain Monte Carlo (MCMC) chains (nchains = 4) and every 1000th tree saved (samplefreq = 1000). Bayesian analyses used the following parameters: nst = 6, rates = invgamma and gamma for SSU and LSU rDNA datasets respectively, ngammacat = 4, the MCMC parameters were left at the default settings, and the priors parameters of the combined dataset were set to ratepr = fixed. Samples of substitution model parameters, and tree and branch lengths were summarised using the parameters 'sump burnin = 3000' and 'sumt burnin = 3000'. These 'burnin' parameters were chosen because the log likelihood scores 'plateaued' well before 3 000 000 replicates in the Bayesian inference analyses.

Neighbour-joining analysis was also performed on a separate dataset containing all SSU rDNA isolates of *Ceratomyxa cardinalis* nov. sp. and the closest related sequence, *C. jonesi*. This dataset was aligned, edited and trimmed as described above, resulting in an alignment of 1,396 bases. Neighbour-joining analysis was conducted using the default parameters to construct trees using PAUP* 4.0b10 (Swofford 2002). The strength of resultant relationships was tested by bootstrap analyses with 10 000 replicates.

Pairwise differences were analysed using PAUP* 4.0b10 to determine total nucleotide distance and percentage differences. Pairwise difference analyses were performed using a dataset containing the new species from this study and the most closely related sequences as determined by BLAST analyses. These datasets were aligned, edited and trimmed as described above. The resulting alignments were 1,476 and 1,289 bases for SSU and LSU rDNA, respectively.

Results

Sample Collection

Between 2005 and 2012, 724 individual apogonids were examined for the presence of *Ceratomyxa* infections from three localities, off Heron Island on the southern Great Barrier Reef, off Lizard Island on the northern Great Barrier Reef and Ningaloo Reef, off Point Cloates, Western Australia (Table I). A total

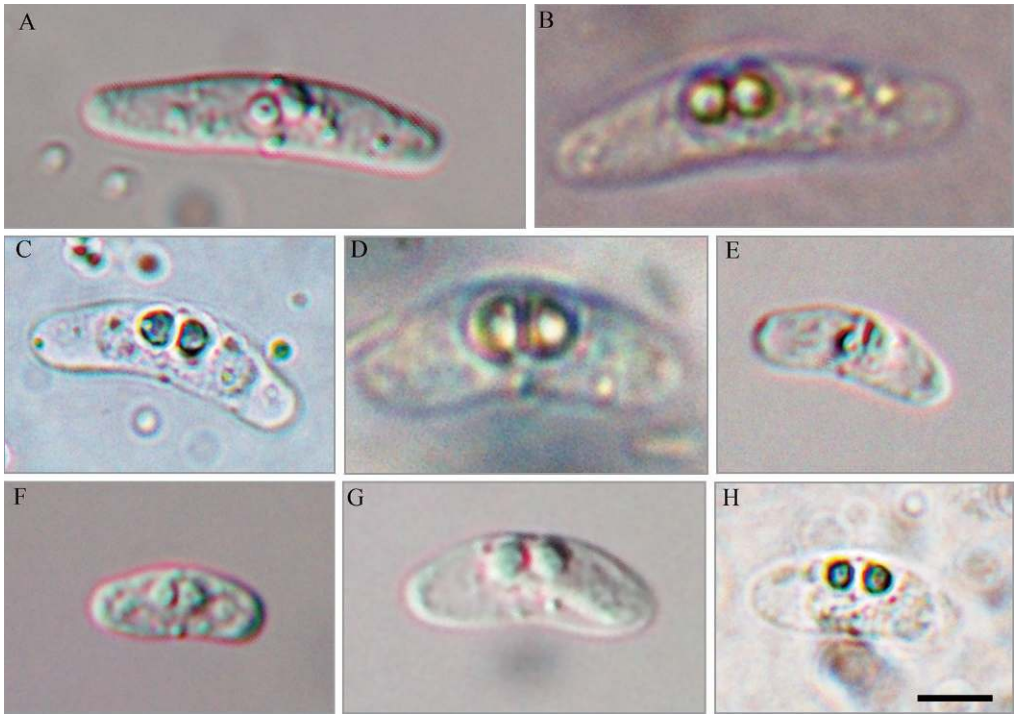

Fig. 1. Photomicrographs of spores of the new *Ceratomyxa* spp.: (**A**) *C. gunterae* sp. nov.; (**B**) *C. rueppellii* sp. nov.; (**C**) *C. cyanosomae* sp. nov.; (**D**) *C. ostorhinchi* sp. nov.; (**E**) *C. heronensis* sp. nov.; (**F**) *C. archamiae* sp. nov; (**G-H**) *C. ireneae* sp. nov. ex (**G**) *Zoramia leptacantha* and (**H**) *Archamia fucata*. Scale bar = 5 μm

of 23 species from nine genera were sampled. This survey identified 234 isolates (22 host/parasite/location combinations) of *Ceratomyxa* infecting the gall bladders of apogonids from all three locations. Overall, this survey found 65% (15/23) of apogonid species and 32% (234/724) of individuals infected with *Ceratomyxa.* Four apogonid species were infected with multiple *Ceratomyxa* species. These infections were identified and described based on morphological and molecular data taken from the same individual host and it was assumed that no mixed congeneric infections were present. No mixed infections were identified in any other hosts.

New Taxa

Class Myxosporea
Order Bivalvulida
Family Ceratomyxidae Doflein, 1899
Genus *Ceratomyxa* Thélohan, 1892

Ceratomyxa gunterae sp. nov. (Figs 1A, 2A, 4–5, Table II–III)

Type host: *Apogon doederleini* Jordan et Snyder, 1901 (Apogonidae).

Type locality: Lagoon, Heron Island (23°26′S, 151°54′E), Great Barrier Reef, Queensland, Australia.

Location in the host: Gall bladder.

Prevalence: 3 of 14 (21%) at Heron Island; 0 of 2 at Lizard Island.

Material deposited: Syntypes – Giemsa-stained, air-dried spores (QM G465540, G465541). GenBank accession number: JX971422.

Etymology: The species *gunterae* is in honour of Dr Nicole Gunter, in recognition of her contribution to the research of Australian *Ceratomyxa* species.

Description: Plasmodia – not observed. Spores – typical of genus *Ceratomyxa.* Mature spores slightly crescent shaped. Posterior angle slightly concave to slightly convex. Valves unequal, smoothly ovoid in lateral view. Suture line straight. Polar capsules subspherical. Mature spore measurements are shown in Table II.

Morphological affinities: *Ceratomyxa gunterae* sp. nov. is morphologically similar in shape and size to *Ceratomyxa heinigerae* Gunter, Whipps et Adlard, 2009, *C. brayi* Gunter et Adlard, 2009, *C. reniforma* Wu, Wu et Dingke, 1993 and *C. diplodae* Lubat, Radujković, Marques et Bouix, 1989. *Ceratomyxa gunterae* sp. nov. can be distinguished from *C. heinigerae*, *C. brayi* and *C. diplodae* on the basis of the size and shape of the polar capsules. *Ceratomyxa gunterae* sp. nov. has larger (1.6–3.7 x 1.4–3 µm) subspherical polar capsules than *C. heinigerae* (1.8–2.4 x 1.6–2.4 µm), *C. brayi* (1.5–2.5 x 1.5–2.5µm) and *C. diplodae* (2.3 x 2 µm) which all have smaller, more spherical polar capsules. *Ceratomyxa diplodae* also has thinner spores (18–22 µm) than *C. gunterae* sp. nov. (17.2–29.6 µm). *Ceratomyxa brayi* can be further distinguished from *C. gunterae* sp. nov. by its triangular spore shape and shorter spores (4.5–6 µm). *Ceratomyxa reniforma* has larger (2.5–3 x 2.5–3µm), spherical polar capsules distinguishing it from *C. gunterae* sp. nov.

Sequence data and molecular affinities: Identical SSU rDNA sequences of 1324 bases were generated from three individual fish for *C. gunterae* sp. nov. (GenBank accession number JX971422). This sequence differs from the aligned sequences of other *Ceratomyxa* species by 16–222 nucleotides (Table III). *Ceratomyxa gunterae* sp. nov. is genetically most similar to *C. archamiae* sp. nov. with 98.8% sequence identity.

Ceratomyxa rueppellii sp. nov. (Fig. 1B, 2B, 4–5, Table II–III)

Type host: *Ostorhinchus rueppellii* (Günther, 1859).

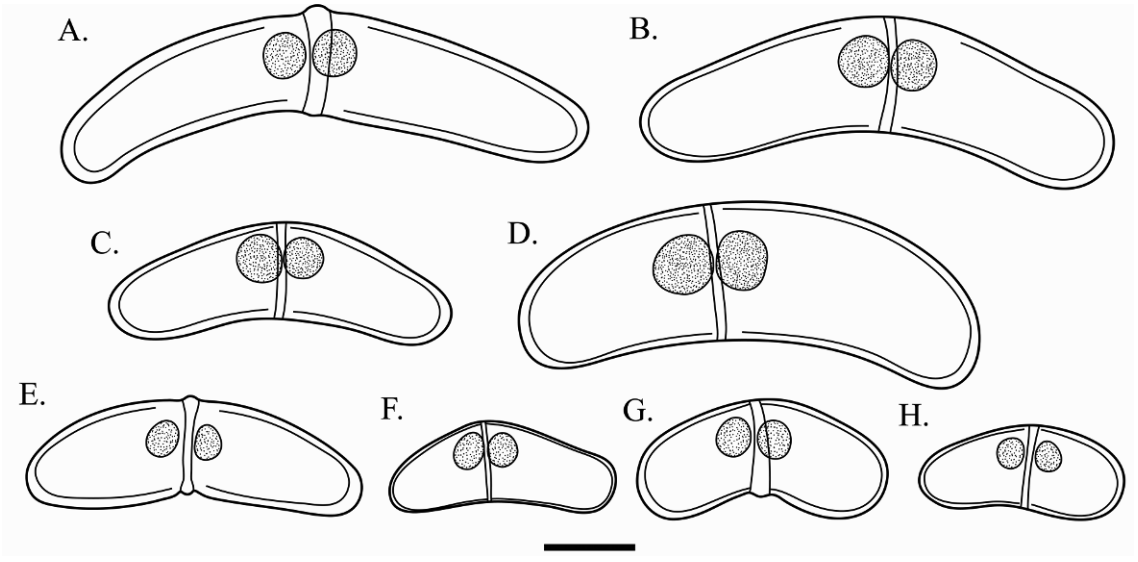

Fig. 2. Illustrations of new *Ceratomyxa* spp.: (**A**) *C. gunterae* sp. nov.; (**B**) *C. rueppellii* sp. nov.; (**C**) *C. cyanosomae* sp. nov.; (**D**) *C. ostorhinchi* sp. nov; (**E**) *C. heronensis* sp. nov.; (**F**) *C. archamiae* sp. nov; (**G**) *C. ireneae* sp. nov.; (**H**) *C. cardinalis* sp. nov. Scale bar = 5µm

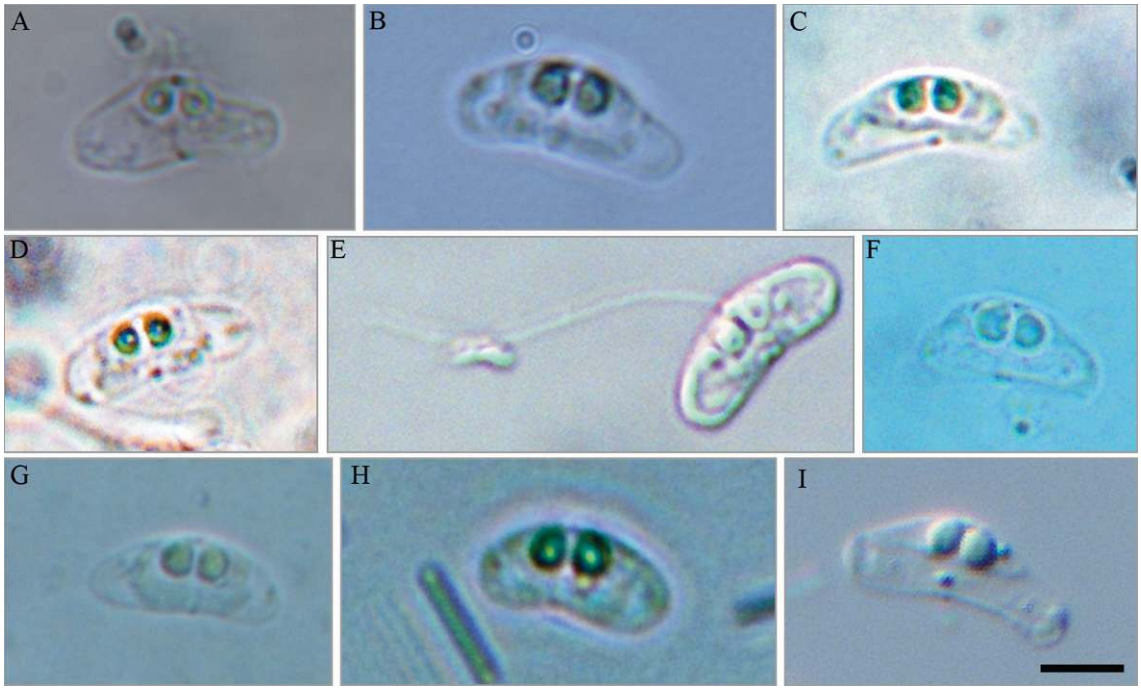

Fig. 3. Photomicrographs of spores of *Ceratomyxa cardinalis* sp. nov. ex: (A) *Cheilodipterus artus* (LI); (B) *C. intermedius* (HI); (C) *C. quinquelineatus* (HI); (D) *C. quinquelineatus* (LI); (E) *Nectamia fusca* (HI); (F) *N. fusca* (LI); (G) *Ostorhinchus cookii* (HI); (H) *O. cyanosoma* (NR); (I) *Zoramia leptacantha* (LI). HI: Heron Island, LI: Lizard Island, NR: Ningaloo Reef. Scale bar = 5µm

Type locality: Off Point Cloates (22°40′S, 113°41′E), Ningaloo Reef, Western Australia, Australia.

Location in the host: Gall bladder.

Prevalence: 6 of 16 (38%).

Material deposited: Syntype – Giemsa-stained, air-dried spores (WAM Z30498). Voucher – Giemsa-stained, air-dried spores (QM G465542 – G465543). GenBank accession number: JX971423.

Etymology: The species epithet *rueppellii* is in reference to the type-host species, *O. rueppellii*.

Description: Plasmodia – Monosporous. Spores – Typical of genus *Ceratomyxa.* Mature spores slightly crescent shaped. Posterior angle slightly concave to slightly convex. Valves almost equal, smoothly ovoid in lateral view. Suture line straight. Polar capsules subspherical. Mature spore measurements are shown in Table II.

Morphological affinities: *Ceratomyxa rueppellii* sp. nov. is morphologically similar in shape and size to *C. nowakae* Gunter, Whipps et Adlard, 2009, *C. heinigerae*, *C. brayi* and *C. reniforma*. *Ceratomyxa rueppellii* sp. nov. can be distinguished from all these species on the basis of polar capsule size and shape (all are spherical). The polar capsules of *C. nowakae* (1.9–2.7 x 1.9–2.8 µm), *C. heinigerae* (1.8–2.4 x 1.6–2.4 µm) and *C. brayi* (1.5–2.5 x 1.5–2.5 µm) are smaller, while *C. reniforma* (2.5–3 x 2.5–3 µm) has, on average, larger polar capsules than *C. rueppellii* sp. nov. (1.9–3.8 x 1.8–3 µm). *Ceratomyxa nowakae* can be further distinguished from *C. rueppellii* sp. nov. by its thinner spores (16.3–23.6 µm) and more concave posterior angle (100–170°). *Ceratomyxa rueppellii* sp. nov. also has longer spores (5.2–7.3 µm) than *C. heinigerae* (4.8–6.3µm) and *C. brayi* (4.5–6µm).

Sequence data and molecular affinities: A single SSU rDNA sequence of 1,389 bases was generated for *C. rueppellii* sp. nov. (GenBank accession number JX971423). The sequence differs from the aligned sequences of other *Ceratomyxa* species by 97–231 nucleotides (Table III). *Ceratomyxa rueppellii* sp. nov. is genetically most similar to *C. cyanosomae* sp. nov. and *C. ostorhinchi* sp. nov. with 93.1% sequence identity.

Ceratomyxa cyanosomae sp. nov. (Fig. 1C, 2C, 4–5, Table II–III)

Type host: *Ostorhinchus cyanosoma* (Bleeker, 1853) (Apogonidae).

Type locality: Lagoon, Lizard Island (14°40′S, 145°27′E), Great Barrier Reef, Queensland, Australia.

Location in the host: Gall bladder.

Prevalence: 1 of 4 (25%) Lizard Island, 0 of 23 Ningaloo Reef.

Material deposited: Syntype – Giemsa-stained, air-dried spores (QM G465544). GenBank accession number: JX971424.

Etymology: The species epithet *cyanosomae* is in reference to the type-host species, *O. cyanosoma*.

Description: Plasmodia – Monosporous or disporous. Spores – Typical of genus *Ceratomyxa.* Mature spores slightly crescent shaped. Posterior angle slightly concave to slightly convex. Valves almost equal, smoothly ovoid in lateral view. Suture line straight. Polar capsules spherical. Mature spore measurements are shown in Table II.

Table II. Morphometrics of new *Ceratomyxa* species. Mean spore dimensions are in µm or degrees (range in parentheses)

***Ceratomyxa* species**	**Host species**	**Locality**	**Length**	**Thickness**	**Sutural Position**	**PC Length**	**PC Width**	**Posterior Angle**
Ceratomyxa gunterae sp. nov. (n = 34)	*Apogon doederleini*	Heron Island	5.8 ± 0.7 (4.4–7.5)	24.4 ± 3.2 (17.2–29.6)	12.7 ± 1.8 (9.8–16.3)	2.7 ± 0.5 (1.6–3.7)	2.2 ± 0.3 (1.4–3)	159.2 ± 12.4 (128.6–185.2)
Ceratomyxa rueppellii sp. nov. (n = 32)	*Ostorhinchus rueppellii*	Ningaloo Reef	6.4 ± 0.5 (5.2–7.3)	23.6 ± 2.5 (17.3–28.3)	11.7 ± 1.5 (8.6–14.8)	2.6 ± 0.4 (1.9–3.8)	2.4 ± 0.3 (1.8–3)	167.8 ± 15.5 (134–196)
Ceratomyxa cyanosomae sp. nov. (n = 30)	*Ostorhinchus cyanosoma*	Lizard Island	6.1 ± 0.8 (5–8)	20 ± 1.8 (16.7–24.2)	10 ± 1.2 (7.9–12.7)	2.5 ± 0.2 (2.1–2.8)	2.3 ± 0.1 (2–2.7)	164.2 ± 12.2 (139.2–186.8)
Ceratomyxa ostorhinchi sp. nov. (n = 30)	*Ostorhinchus aureus*	Ningaloo Reef	6.8 ± 0.8 (5.3–8.6)	24.2 ± 1.6 (21.2–27.5)	12 ± 1.3 (9.2–14.7)	3.3 ± 0.5 (2.4 – 4.6)	2.5 ± 0.4 (1.7–3.2)	162.6 ± 18.5 (135–204)
Ceratomyxa heronensis sp. nov. (n = 24)	*Archamia fucata*	Heron Island	5.2 ± 0.6 (4–6.2)	12.2 ± 1.3 (10.3–15.1)	6.4 ± 0.8 (4.5–7.9)	2.3 ± 0.3 (1.8–3)	1.9 ± 0.2 (1.5–2.4)	169.1 ± 14.1 (132.4–193.9)
Ceratomyxa archamiae sp. nov. (n = 25)	*Archamia fucata*	Heron Island	4.9 ± 0.4 (4.2–5.7)	10.9 ± 1 (8.8–12.4)	5.8 ± 0.7 (4.2–6.9)	2.1 ± 0.2 (1.6–2.5)	1.8 ± 0.2 (1.1–2.1)	171 ± 11.2 (151.4–195.9)
Ceratomyxa ireneae sp. nov. (n = 30)	*Zoramia viridiventer*	Lizard Island	6.1 ± 0.6 (5.2–8.3)	15 ± 1.6 (11.4–17.8)	7.8 ± 1 (6–9.4)	2.7 ± 0.3 (2.1–3.5)	2.2 ± 0.3 (1.7–3)	156.5 ± 20.1 (89.2–188)
Ceratomyxa ireneae sp. nov. (n = 30)	*Archamia fucata*	Lizard Island	5.2 ± 0.4 (4.5–6.2)	14.5 ± 1.4 (12.2–17.3)	7.2 ± 0.7 (5.9 –8.7)	2 ± 0.2 (1.6–2.3)	1.7 ± 0.1 (1.5–2)	173.5 ± 6.4 (160.3–183.8)
Ceratomyxa cardinalis sp. nov. (n = 62)	*Nectamia fusca*	Heron Island	5.5 ± 0.7 (4.3–7.3)	12.2 ± 1 (10–14.4)	6.3 ± 0.7 (4.8–8.4)	2.4 ± 0.3 (1.9–3.3)	1.9 ± 0.2 (1.4 –2.6)	172.4 ± 11.5 (136.6–191.7)
Ceratomyxa cardinalis sp. nov. (n = 57)	*Nectamia fusca*	Lizard Island	5.2 ± 0.5 (4–6.8)	12.1 ± 1.1 (9.5–14.8)	6.2 ± 0.6 (4.6–7.3)	2.2 ± 0.2 (1.7–2.6)	1.8 ± 0.2 (1.3–2.2)	170.8 ± 7 (155.2–186.2)
Ceratomyxa cardinalis sp. nov. (n = 33)	*Cheilodipterus artus*	Lizard Island	5.2 ± 0.6 (4.2–6.5)	11.9 ± 1 (10.1–14.6)	6.3 ± 0.8 (4.9–8)	2.5 ± 0.3 (1.9–3)	2 ± 0.3 (1.5–2.6)	169.8 ± 8.4 (152.3–185)
Ceratomyxa cardinalis sp. nov. (n = 14)	*Cheilodipterus intermedius*	Heron Island	5.3 ± 0.5 (4.7–6.4)	13.8 ± 0.6 (12.9–15.3)	6.9 ± 0.8 (5.6–8.1)	2.3 ± 0.2 (1.8–2.6)	1.8 ± 0.2 (1.5–2.1)	159.4 ± 11.7 (134–173)
Ceratomyxa cardinalis sp. nov. (n = 11)	*Cheilodipterus quinquelineatus*	Heron Island	4.9 ± 0.3 (4.3–5.4)	12.5 ± 0.9 (10.9–13.7)	6.1 ± 0.6 (5.3–7.1)	2.2 ± 0.2 (2 –2.6)	1.8 ± 0.1 (1.6–2)	172.9 ± 10.6 (152–187.8)
Ceratomyxa cardinalis sp. nov. (n = 61)	*Cheilodipterus quinquelineatus*	Lizard Island	4.7 ± 0.4 (3.8–6)	13.1 ± 1.4 (9.7–16.5)	6.5 ± 0.8 (4.8–8.3)	2 ± 0.2 (1.5–2.3)	1.7 ± 0.2 (1.3–2.2)	164.9 ± 10.3 138.1–187.6)
Ceratomyxa cardinalis sp. nov. (n = 141)	*Ostorhinchus cookii*	Heron Island	4.8 ± 0.5 (3.9–6.4)	12.3 ± 1.4 (8.6–15.4)	6.4 ± 0.7 (4.3–7.7)	2.1 ± 0.3 (1.4–2.8)	1.9 ± 0.2 (1.1–2.4)	172.7 ± 9.5 (132.4–189.5)
Ceratomyxa cardinalis sp. nov. (n = 28)	*Ostorhinchus cyanosoma*	Ningaloo Reef	4.6 ± 0.3 (4.2–5.2)	9.7 ± 0.8 (8.2–11.6)	4.8 ± 0.4 (4.1–5.7)	2.1 ± 0.2 (1.7–2.6)	1.7 ± 0.2 (1.3–2.3)	158.2 ± 13.7 (132.5–183.3)
Ceratomyxa cardinalis sp. nov. (n = 64)	*Zoramia leptacantha*	Lizard Island	5.5 ± 0.4 (4.5–6.5)	13.1 ± 1.2 (10.5–15.7)		2.6 ± 0.2 (2.2–3)	2.3 ± 0.2 (1.9– 2.7)	166.1 ± 10.8 (135.3–186.7)

PC – Polar capsule.

Table III. SSU rDNA nucleotide differences (below oblique) and percentage similarity (above oblique) of isolates from new *Ceratomyxa* species (shown in bold) and related species

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1. *Ceratomyxa cardinalis* n. sp. JX971431		99.9	99.9	99.9	99.9	99.9	99.9	99.7	99.6	99.6	96.9	96.6	96.3	96.3	96.1	96.1	95.8	97	95.9	95.8	95.7	95	84.8	85.5	84.4
2. *Ceratomyxa cardinalis* n. sp. JX971433	1		99.9	99.9	99.9	99.9	99.8	99.6	99.6	99.6	96.9	96.6	96.3	96.2	96	96	95.8	96.9	95.8	95.7	95.7	94.9	84.7	85.5	84.3
3. *Ceratomyxa cardinalis* n. sp. JX971432	1	2		99.9	99.9	99.9	99.8	99.6	99.6	99.6	96.9	96.6	96.3	96.2	96	96	95.8	96.9	95.8	95.8	95.7	95.1	84.8	85.6	84.5
4. *Ceratomyxa cardinalis* n. sp. JX971436	1	2	2		99.9	99.9	99.8	99.6	99.6	99.6	96.9	96.6	96.3	96.2	96	96	95.8	96.9	95.8	95.7	95.7	94.9	84.8	85.6	84.5
5. *Ceratomyxa cardinalis* n. sp. JX971434	1	2	2	2		99.9	99.8	99.6	99.6	99.6	97	96.7	96.4	96.3	96.2	96.1	95.9	96.9	96	95.7	95.7	95.1	84.8	85.5	84.4
6. *Ceratomyxa cardinalis* n. sp. JX971435	1	2	2	2	2		99.9	99.6	99.7	99.6	96.9	96.6	96.4	96.3	96	96	95.8	96.9	96	95.7	95.7	95.1	84.7	85.6	84.3
7. *Ceratomyxa cardinalis* n. sp. JX971439	2	3	3	3	3	1		99.6	99.6	99.5	96.8	96.5	96.3	96.3	96	95.9	95.7	96.9	95.9	95.7	95.6	95	84.7	85.6	84.3
8. *Ceratomyxa cardinalis* n. sp. JX971438	4	5	5	5	5	5	6		99.9	99.9	96.6	96.4	96.1	96	95.8	95.8	95.9	96.7	95.6	95.5	95.5	94.7	84.8	85.3	84.1
9. *Ceratomyxa cardinalis* n. sp. JX971440	5	6	6	6	6	4	5	1		99.9	96.6	96.3	96.1	96.1	95.8	95.7	95.8	96.6	95.7	95.5	95.4	94.8	84.7	85.3	84.1
10. *Ceratomyxa cardinalis* n. sp. JX971437	5	6	6	6	6	6	7	1	2		96.7	96.4	96.1	96	95.9	95.7	95.8	96.6	95.6	95.5	95.4	94.7	84.9	85.3	84.2
11. *Ceratomyxa archamiae* n. sp. JX971428	41	42	42	42	40	42	43	45	46	44		98.8	98.6	98.6	97.4	95.8	95.5	97.4	97.1	96.1	96.1	95.4	84	85.2	84.1
12. *Ceratomyxa gunterae* n. sp. JX971422	45	46	46	46	44	46	47	49	50	49	16		98.6	98.6	97.1	96	95.3	97.4	97.7	96.5	96.5	96.2	84.1	85.4	84.1
13. *Ceratomyxa heronensis* n. sp. JX971426	49	50	50	50	48	48	49	53	52	53	19	19		99.9	96.8	95.4	95.1	97.1	97.4	96	96	95.9	83.5	84.8	83.4
14. *Ceratomyxa heronensis* n. sp. JX971427	50	51	51	51	49	49	50	54	53	54	18	18	1		96.9	95.5	95.2	97.2	97.4	95.9	95.9	95.8	83.5	84.9	83.4
15. *Ceratomyxa ernsti* FJ204247	52	53	53	53	51	53	53	56	57	55	34	38	42	41		95.5	95	96.9	97.3	96.9	96.8	95.3	84.4	85.3	84.2
16. *Ceratomyxa talboti* EU440368	53	54	54	54	52	54	55	57	58	58	56	54	62	61	61		95.6	95.2	95.2	94.6	94.5	93.9	83.9	84.9	83.9
17. *Ceratomyxa cribbi EU440367*	56	57	57	57	55	57	58	55	56	56	60	63	66	65	68	60		94.3	94.1	93.9	93.8	93.2	83.5	84.3	83.4
18. *Ceratomyxa jonesi* FJ204250	40	41	41	41	41	41	42	44	45	45	35	34	38	37	41	64	77		96.8	96.9	96.8	95.4	84	85.3	84.1
19. *Ceratomyxa dennisi* EU440358	55	56	56	56	54	54	55	59	58	59	39	31	35	34	36	64	79	42		97	97	95.6	84	85.3	83.8
20. *Ceratomyxa ireneae* n. sp. JX971430	56	57	56	57	57	57	58	60	61	61	52	46	53	54	41	73	82	41	40		99.8	95.6	84.3	85.3	84.2
21. *Ceratomyxa ireneae* n. sp. JX971429	57	58	57	58	58	58	59	61	62	62	52	46	53	54	42	74	83	42	40	3		95.5	84.3	85.4	84.2
22. *Ceratomyxa diamanti* FJ204246	67	68	66	68	66	66	67	71	70	71	61	50	55	56	63	82	92	61	59	59	60		83.9	84.8	83.4
23. *Ceratomyxa rueppellii* n. sp. JX971423	213	214	212	212	213	214	214	213	214	212	224	222	230	231	218	225	231	223	224	220	219	226		93.1	93.1
24. *Ceratomyxa cyanosomae* n. sp. JX971424	201	201	200	200	201	200	200	205	204	204	205	202	210	209	203	209	219	203	204	204	202	211	97		94.5
25. *Ceratomyxa ostorhinchi* n. sp. JX971425	219	220	218	218	219	220	220	223	224	222	223	222	233	232	221	226	234	223	227	221	221	233	97	77	

Morphological affinities: *Ceratomyxa cyanosomae* sp. nov. is morphologically similar in shape and size to *C. nowakae*, *C. gleesoni* Gunter et Adlard, 2009, *C. diplodae* and *C. reniforma*. *Ceratomyxa cyanosomae* sp. nov. can be distinguished from all of these species by the size of its polar capsules (2.1–2.8 x 2–2.7 µm). *Ceratomyxa nowakae* (1.9–2.7 x 1.9–2.8 µm), *C. gleesoni* (1.5–2.5 x 1.5–2.5 µm) and *C. diplodae* (2.25 x 2 µm) have smaller polar capsules, while *C. reniforma* (2.5–3 x 2.5–3µm) has larger more spherical polar capsules. *Ceratomyxa nowakae* can be further distinguished from *C. cyanosomae* sp. nov. by the more acute posterior angle of its spore. *Ceratomyxa reniforma* has thicker spores (19–27 µm) than *C. cyanosomae* sp. nov. (16.7–24.2 µm). The spores of *C. cyanosomae* sp. nov. are generally larger than those of *C. gleesoni*, although the spore measurements overlap, the ranges are larger in all instances.

Sequence data and molecular affinities: A single SSU rDNA sequence of 1377 bases was generated for *C. cyanosomae* n. sp. nov. (GenBank accession number JX971424). The sequence differs from the aligned sequences of other *Ceratomyxa* species by 77–219 nucleotides (Table III). *Ceratomyxa cyanosomae* sp. nov. is genetically most similar to *C. ostorhinchi* sp. nov. with 94.5% sequence identity.

Ceratomyxa ostorhinchi sp. nov. (Fig. 1D, 2D, 4–5, Table II–III)

Type host: *Ostorhinchus aureus* (Lacépède, 1802) (Apogonidae).

Type locality: Off Point Cloates (22°40′S, 113°4′E), Ningaloo Reef, Western Australia, Australia.

Location in the host: Gall bladder.

Prevalence: 1 of 14 (7%).

Material deposited: Syntype – Giemsa-stained, air-dried spores (WAM Z30499). DNA voucher (QM G465545). GenBank accession number: JX971425.

Etymology: The species epithet *ostorhinchi* is in reference to the genus of the type-host, *Ostorhinchus*.

Description: Plasmodia – Disporous. Spores – Typical of genus *Ceratomyxa.* Mature spores slightly crescent shaped. Posterior angle slightly concave to slightly convex. Valves almost equal, smoothly ovoid in lateral view. Suture line straight. Polar capsules subspherical. Mature spore measurements are shown in Table II.

Morphological affinities: *Ceratomyxa ostorhinchi* sp. nov. is morphologically similar in shape and size to *C. cottoidii* Reed, Basson, Van As et Dyková, 2007, *C. yokoyamai* Gunter et Adlard, 2009 and *C. capricornensis* Gunter et Adlard, 2008. *Ceratomyxa ostorhinchi* sp. nov. can be distinguished from all species above by its larger polar capsules. *Ceratomyxa cottoidii* can be further distinguished from *C. ostorhinchi* sp. nov. by its longer (6.5–8 µm) and thinner (17–22 µm) spores, while *C. yokoyamai* and *C. capricornensis* both have shorter (4.5–6.5 and 4.8–8.1µm, respectively) and thicker (20.5–31 and 21.6–36.7µm, respectively) spores.

Sequence data and molecular affinities: A single SSU rDNA sequence of 1,393 bases was generated for *C. ostorhinchi* sp. nov. (GenBank accession number JX971425). The sequence differs from the aligned sequences of other *Ceratomyxa* species by 77–234 nucleotides (Table III). *Ceratomyxa ostorhinchi* sp. nov. is genetically most similar to *C. cyanosomae* sp. nov. with 94.5% sequence identity.

Ceratomyxa heronensis sp. nov. (Fig. 1E, 2E, 4–5, Table II–III)

Type host: *Archamia fucata* (Cantor, 1849).

Type locality: Lagoon, Heron Island (23°26′S, 151°54′E), Great Barrier Reef, Queensland, Australia.

Location in the host: Gall bladder.

Prevalence: 2 of 20 (10%) at Heron Island; 0 of 26 at Lizard Island.

Material deposited: Syntype – Giemsa-stained, air-dried spores (QM G465546). GenBank accession number: JX971426, JX971427.

Etymology: The species epithet *heronensis* refers to the locality where this species was recovered.

Description: Plasmodia – not observed. Spores – Typical of genus *Ceratomyxa*. Mature spores slightly crescent shaped. Posterior angle slightly concave to slightly convex. Valves unequal, smoothly ovoid in lateral view. Suture line straight. Polar capsules subspherical. Mature spore measurements are shown in Table II.

Morphological affinities: *Ceratomyxa heronensis* sp. nov. is morphologically similar in shape and size to *C. cardinalis* sp. nov., *C. ireneae* sp. nov., *C. castigata* Meglitsch, 1960, *Ceratomyxa dennisi* Gunter et Adlard, 2008, *C. talboti* Gunter et Adlard, 2008 and *C. bryanti* Gunter et Adlard, 2008. *Ceratomyxa heronensis* sp. nov. has larger polar capsules (1.8–3 x 1.5–2.4 µm) than *C. dennisi* (1.2–2.3 x 1.1–2.3 µm), *C. talboti* (1–2.3 x 1.3–2.6 µm) and *C. bryanti* (1.5–2 x 1.3–1.9 µm). *Ceratomyxa dennisi* and *C. bryanti* can be further distinguished by their thicker spores (9.3–17.3 and 10.2–19.4 µm, respectively). *Ceratomyxa heronensis* sp. nov. has shorter spores (4–6.2 µm) than *C. castigata* (5.1–6.9 µm). *Ceratomyxa ireneae* sp. nov. has thicker spores than *C. heronensis* sp. nov. While there is morphological overlap in all measurements between *C. heronensis* sp. nov. and *C. cardinalis* sp. nov. the ranges are greater for *C. cardinalis* sp. nov.

Sequence data and molecular affinities: Two SSU rDNA sequences of 1,326 bases were generated from three individual fish for *C. heronensis* sp. nov. (GenBank accession numbers JX971426 and JX971427). There is one nucleotide difference (99.9% sequence identity) between the two SSU rDNA sequences. These sequences differ from the aligned sequences of other *Ceratomyxa* species by 18–233 nucleotides (Table III). *Ceratomyxa heronensis* sp. nov. is genetically most similar to *C. archamiae* sp. nov. and *C. gunterae* sp. nov. with 98.6% maximum sequence identity.

Ceratomyxa archamiae sp. nov. (Fig. 1F, 2F, 4–5, Table II–III)

Type host: *Archamia fucata* (Cantor, 1849).

Type locality: Lagoon, Heron Island (23°26´S, 151°54´E), Great Barrier Reef, Queensland, Australia.

Location in the host: Gall bladder.

Prevalence: 2 of 20 (10%) at Heron Island; 0 of 26 at Lizard Island.

Material deposited: *Syntype* – Giemsa-stained, air-dried spores (QM G465547). GenBank accession number: JX971428.

Etymology: The species epithet *archamiae* is in reference to the genus of the type-host, *Archamia*.

Description: Plasmodia – not observed. Spores – Typical of genus *Ceratomyxa.* Mature spores slightly crescent shaped. Posterior angle slightly concave to slightly convex. Valves unequal, smoothly ovoid in lateral view. Suture line straight. Polar capsules subspherical. Mature spore measurements are shown in Table II.

Morphological affinities: *Ceratomyxa archamiae* sp. nov. is morphologically similar in shape and size to *C. arripica* Su et White, 1994, *C. americana* Wierzbicka, 1987, and *C. ernsti* Gunter, Whipps et Adlard, 2009. *Ceratomyxa archamiae* sp. nov. can be distinguished from all species above based on the shape of its polar capsules, subspherical rather than spherical. *Ceratomyxa archamiae* sp. nov. also has thicker spores (8.8–12.4 µm) than *C. americana* (8.8–11.2 µm). *Ceratomyxa americana* also differs to *C. archamiae* sp. nov. on the basis of spore shape, *C. americana* has spores with pointed ends which have a downwards direction. *Ceratomyxa ernsti* has longer (4.7–6.8 µm) and thicker (9.5–14.8 µm) spores than *C. archamiae* sp. nov. (4.2–5.7 and 8.8–12.4 µm, respectively).

Sequence data and molecular affinities: Identical SSU rDNA sequences of 1331 bases were generated from two individual fish for *C. archamiae* sp. nov. (GenBank accession number JX971428). This sequence differs from the aligned sequences of other *Ceratomyxa* species by 16–224 nucleotides (Table III). *Ceratomyxa archamiae* sp. nov. is genetically most similar to *C. gunterae* sp. nov. with 98.8% sequence identity.

Ceratomyxa ireneae sp. nov. (Fig. 1G-H, 2G, 4–5, Table II–III)

Type host: *Zoramia viridiventer* Greenfield, Langston et Randall, 2005 (Apogonidae).

Other hosts: *Archamia zosterophora* (Bleeker, 1856), *Archamia fucata* (Cantor, 1849).

Type locality: Lagoon, Lizard Island (14°40´S, 145°27´E), Great Barrier Reef, Queensland, Australia.

Location in the host: Gall bladder.

Prevalence: 8 of 74 (11%) in *Z. viridiventer*; 2 of 2 (100%) in *A. zosterophora*; 13 of 26 (50%) at Lizard Island, and 0 of 20 at Heron Island in *A. fucata*.

Material deposited: Syntype – Giemsa-stained, air-dried spores (QM G465548–G465549 ex *Z. viridiventer*). GenBank accession number: JX971429, JX971430.

Etymology: The species epithet *ireneae* is in honour of Mrs Irene Heiniger in recognition of her support and encouragement of this work.

Description: Plasmodia – Monosporous or disporous. Spores – Typical of genus *Ceratomyxa.* Mature spores slightly crescent shaped. Posterior angle concave to slightly convex. Valves almost equal, smoothly ovoid in lateral view. Suture line straight. Polar capsules subspherical to pyriform. Mature spore measurements are shown in Table II.

Morphological affinities: *Ceratomyxa ireneae* sp. nov. is morphologically similar in shape and size to *C. bassoni* Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008, *C. stridensi* Abd El-Monem, Bayoumy et Hassanein, 2005, *C. kenti* Gunter et Adlard, 2008, *C. jonesi* Gunter, Whipps et Adlard, 2009, *C. talboti* Gunter et Adlard, 2008 and *C. cribbi* Gunter et Adlard, 2008. *Ceratomyxa bassoni* can be distinguished from *C. ireneae* sp. nov. by its thicker spore (15–20 µm) and larger polar capsules (3–4 x 2–3 µm). *Ceratomyxa stridensi* can be distinguished from *C. ireneae* sp. nov. by its smaller (1.9–2.3 µm), spherical polar capsules. *Ceratomyxa ireneae* sp. nov. has longer spores (4.5–8.3 µm) and larger polar capsules (1.6–3.5 x 1.5–3 µm) than *C. kenti* (4.1–5.8 µm and 1.2–2.2 x 1–1.9 µm, respectively). *Ceratomyxa ireneae* sp. nov. has longer spores than *C. cribbi* (3.5–5.6 µm). *Ceratomyxa jonesi* has thinner spores (11.2–16.5 µm) than *C. ireneae* sp. nov. (11.4–17.8 µm). *Ceratomyxa ireneae* sp. nov. has longer and thicker spores and larger polar capsules than *C. talboti* (4.1–6.8, 9.9–17.1 µm and 1.3–2.6 x 1–2.3 µm, respectively).

Sequence data and molecular affinities: Two SSU rDNA sequences were generated for *C. ireneae* sp. nov. Identical SSU rDNA sequences of 1322 bases were generated from two fish species, *Z. viridiventer* and *A. zosterophora* (three isolates from individual fish with 100% identity) (GenBank accession number JX971429). Identical SSU rDNA sequences of 1322 bases were also generated from two individual *A. fucata* hosts (GenBank accession number JX971430). There are 3 nucleotides difference (99.8% sequence identity) between the two SSU rDNA sequences. This sequence differs from the aligned sequences of other *Ceratomyxa* species by 40–221 nucleotides (Table III). *Ceratomyxa ireneae* sp. nov. is genetically most similar to *C. dennisi* with 97% sequence identity.

Ceratomyxa cardinalis sp. nov. (Fig. 2H, 3–6, Table II–V)

Type host: *Cheilodipterus artus* Smith, 1961

Type locality: Lagoon, Lizard Island (14°40´S, 145°27´E), Great Barrier Reef, Queensland, Australia.

Location in the host: Gall bladder.

Prevalence: 9 of 13 (70%) Lizard Island.

Other hosts and Localities: *Apogonidae – Apogon doederleini* Jordan et Snyder, 1901, 1 of 14 (7%) at Heron Island, 1 of 2 (50%) at Lizard Island; *Cheilodipterus intermedius* Gon, 1993, 4 of 5 (80%) at Heron Island, 1 of 19 (5%) at Lizard Island, 0 of 1 Ningaloo Reef; *Cheilodipterus quinquelineatus* Cuvier, 1828, 12 of 28 (43%) at Heron Island, 16 of 34 (47%) at Lizard Island; *Nectamia fusca* (Quoy et Gaimard, 1825), 20 of 21 (95%) at Lizard Island; 10 of 32 (31%) at Heron Island;

Table IV. Individual isolate information and corresponding GenBank accession number for SSU rDNA sequences generated for *C. cardinalis* sp. nov.

GenBank Accession Number	Host species (# of isolates)	Location
JX971431	*Apogon doederleini* (1)	Heron Island
	Cheilodipterus artus (1)	Lizard Island
	Cheilodipterus intermedius (1)	Lizard Island
	Cheilodipterus intermedius (3)	Heron Island
	Cheilodipterus quinquelineatus (2)	Lizard Island
	Ostorhinchus aureus (1)	Ningaloo Reef
	Ostorhinchus cyanosoma (1)	Lizard Island
	Ostorhinchus cyanosoma (4)	Ningaloo Reef
	Zoramia leptacantha (2)	Lizard Island
JX971432	*Ostorhinchus cookii* (1)	Heron Island
	Ostorhinchus cookii (1)	Lizard Island
	Ostorhinchus properuptus (1)	Lizard Island
JX971433	*Apogon doederleini* (1)	Lizard Island
	Ostorhinchus rubrimacula (1)	Lizard Island
JX971434	*Cheilodipterus quinquelineatus* (1)	Lizard Island
JX971435	*Cheilodipterus quinquelineatus* (1)	Lizard Island
JX971436	*Cheilodipterus quinquelineatus* (2)	Heron Island
JX971437	*Cheilodipterus quinquelineatus* (1)	Heron Island
JX971438	*Nectamia fusca* (3)	Lizard Island
JX971439	*Nectamia fusca* (4)	Heron Island
JX971440	*Ostorhinchus cookii* (1)	Heron Island

Ostorhinchus aureus (Lacépède, 1802), 1 of 14 (7%) at Ningaloo Reef; *Ostorhinchus cookii* (Macleay, 1881), 19 of 35 (54%) at Heron Island, 4 of 8 (50%) at Lizard Island, 0 of 2 at Ningaloo Reef; *Ostorhinchus cyanosoma* (Bleeker, 1853), 1 of 4 (25%) at Lizard Island, 11 of 23 (48%) at Ningaloo Reef; *Ostorhinchus properuptus* (Whitley, 1964), 2 of 9 (22%) Lizard Island; *Ostorhinchus rubrimacula* (Randall et Kulbicki, 1998), 24 of 29 (83%) at Lizard Island; *Zoramia leptacantha* (Bleeker, 1856), 37 of 269 (14%) at Lizard Island.

Material deposited: Syntypes – Giemsa-stained, air-dried spores (QM G465550). Vouchers – Giemsa-stained, air-dried spores (G465551–G465563). DNA vouchers (QM G465564–G465566, G465622–G465624). GenBank accession numbers: JX971431–JX971440 for SSU and JX971441–JX971443 for LSU rDNA.

Etymology: The species epithet *cardinalis* is in reference to the common name of the type-host family, Cardinalfishes (Apogonidae).

Description: Plasmodia – Monosporous, disporous or polysporous. Spores – Typical of genus *Ceratomyxa*. Mature spores slightly crescent shaped. Posterior angle slightly concave to slightly convex. Valves unequal, smoothly ovoid in lateral view. Suture line straight. Polar capsules subspherical to spherical. Mature spore measurements are shown in Table II.

Table V. LSU rDNA nucleotide differences (below oblique) and percentage similarity (above oblique) of isolates from *Ceratomyxa cardinalis* sp. nov. (shown in bold) and all other *Ceratomyxa* spp. with LSU rDNA sequences

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1. *Ceratomyxa cardinalis* n. sp. JX971443		99.7	99.1	64.2	61.6	61	59.4	57.8
2. *Ceratomyxa cardinalis* n. sp. JX971442	3		99	64.1	61.6	60.8	59.5	57.7
3. *Ceratomyxa cardinalis* n. sp. JX971441	10	11		64.1	61.7	60.6	59.2	57.4
4. *Ceratomyxa* sp. FJ417048	412	413	413		56.7	54.2	57.1	54.1
5. *Ceratomyxa* sp. FJ417050	447	447	446	505		63.9	55	60
6. *Ceratomyxa* sp. FJ417049	477	479	481	562	428		53.5	60.1
7. *Ceratomyxa vikrami* FJ417056	497	496	500	523	546	584		54.2
8. *Ceratomyxa shasta* FJ981818	501	502	505	545	465	484	560	

Morphological affinities: *Ceratomyxa cardinalis* sp. nov. is morphologically similar in shape and size to *C. heronensis* sp. nov., *C. bryanti* Gunter et Adlard, 2008, *C. dennisi* Gunter et Adlard, 2008, *C. talboti* Gunter et Adlard, 2008, *C. jonesi* Gunter, Whipps et Adlard, 2009 and *C. hooperi* Gunter et Adlard, 2009. *Ceratomyxa cardinalis* sp. nov. can be distinguished from *C. bryanti* (1.5–2 x 1.3–1.9 µm), *C. dennisi* (1.2–2.3 x 1.1–2.3 µm) and *C. hooperi* (1–2 x 1–2 µm) by its larger polar capsules (1.4–3.3 x 1.1–2.7µm). *Ceratomyxa bryanti* and *C. dennisi* can be further distinguished from *C. cardinalis* sp. nov. by their thicker spores (10.2–19.4 µm and 9.3–17.3 µm, respectively). *Ceratomyxa jonesi* and *C. talboti* have spores with a greater convex posterior angle (148–199° and 136–202°) than *C. cardinalis* sp. nov. (132.4–191.7°). *Ceratomyxa talboti* also has pyriform polar capsules whereas *C. cardinalis* sp. nov. has spherical to subspherical polar capsules. Morphological distinctions between *C. cardinalis* sp. nov. and *C. heronensis* sp. nov. are listed above.

Sequence data and molecular affinities: A total of 34 SSU rDNA sequences of 1391– 1435 bases were generated from 11 fish species (34 isolates from 17 possible host/parasite/location combinations) for *C. cardinalis* sp. nov. Several iden-

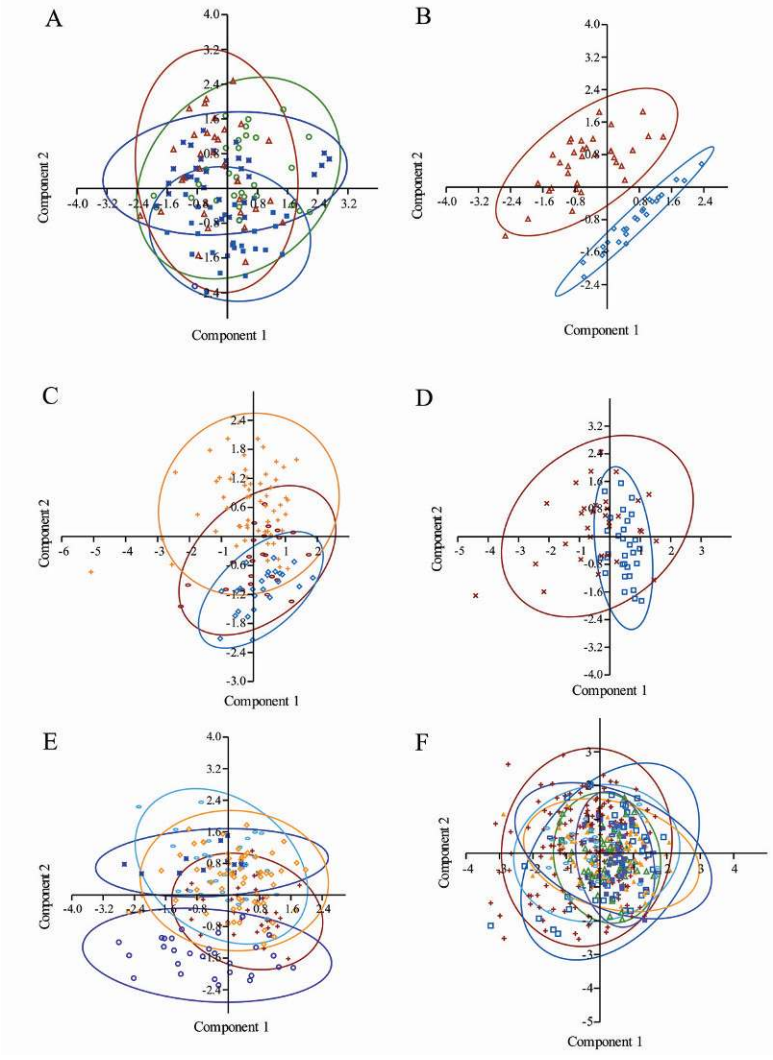

Fig. 4. Principal component analyses with 95% confidence ellipses. (**A**) *Ceratomyxa gunterae* sp. nov. (triangles), *C. rueppellii* sp. nov. (circles), *C. ostorhinchi* sp. nov. (stars), *C. cyanosomae* sp. nov. (filled squares); (**B**) *C. gunterae* sp. nov. (triangles), *C. archamiae* sp. nov. (diamonds); (**C**) *C. heronensis* sp. nov. (ovals), *C. archamiae* sp. nov. (diamonds), *C. ireneae* sp. nov. ex *Z. viridiventer* and *A. fucata* (crosses); (**D**) *C. ireneae* sp. nov. ex *Z. viridiventer* (crosses) and ex *A. fucata* (squares); (**E**) *C. cardinalis* sp. nov. from host isolates with corresponding SSU rDNA sequences of 100% identity (GenBank accession JX971431): ex *C. intermedius* from Heron Island (stars), ex *C. artus* (crosses), ex *C. quinquelineatus* from Lizard Island (ovals), ex *O. cyanosoma* from Ningaloo Reef (circles), and ex *Z. leptacantha* (diamonds); (**F**) *C. cardinalis* sp. nov. ex all combined isolates of GenBank number JX971431 (shown separately in (E)) (crosses), ex *O. cookii* from Heron Island (GenBank number JX971440) (filled squares), ex *O. cookii* from Lizard Island (GenBank number JX971432) (squares), ex *N. fusca* from Lizard Island (GenBank number JX971438) (triangles), ex *N. fusca* from Heron Island (GenBank number JX971439) (filled triangles), ex *C. quinquelineatus* from Heron Island (rectangles), and ex *C. quinquelineatus* from Lizard Island (GenBank number JX971435) (ovals)

tical sequences (0% sequence variation) were generated from multiple host isolates and as such only consensus sequences of these isolates were used in phylogenetic analyses (total of 10 distinct sequences). Individual SSU rDNA isolate information listing host species and geographical location for specific GenBank accession numbers are shown in Table IV. There is 1–7 nucleotides difference (99.5–99.9% sequence identity) between the 10 SSU rDNA sequences generated for *C. cardinalis* sp. nov. These sequences differ from the aligned SSU rDNA sequences of other *Ceratomyxa* species by 40–224 nu-

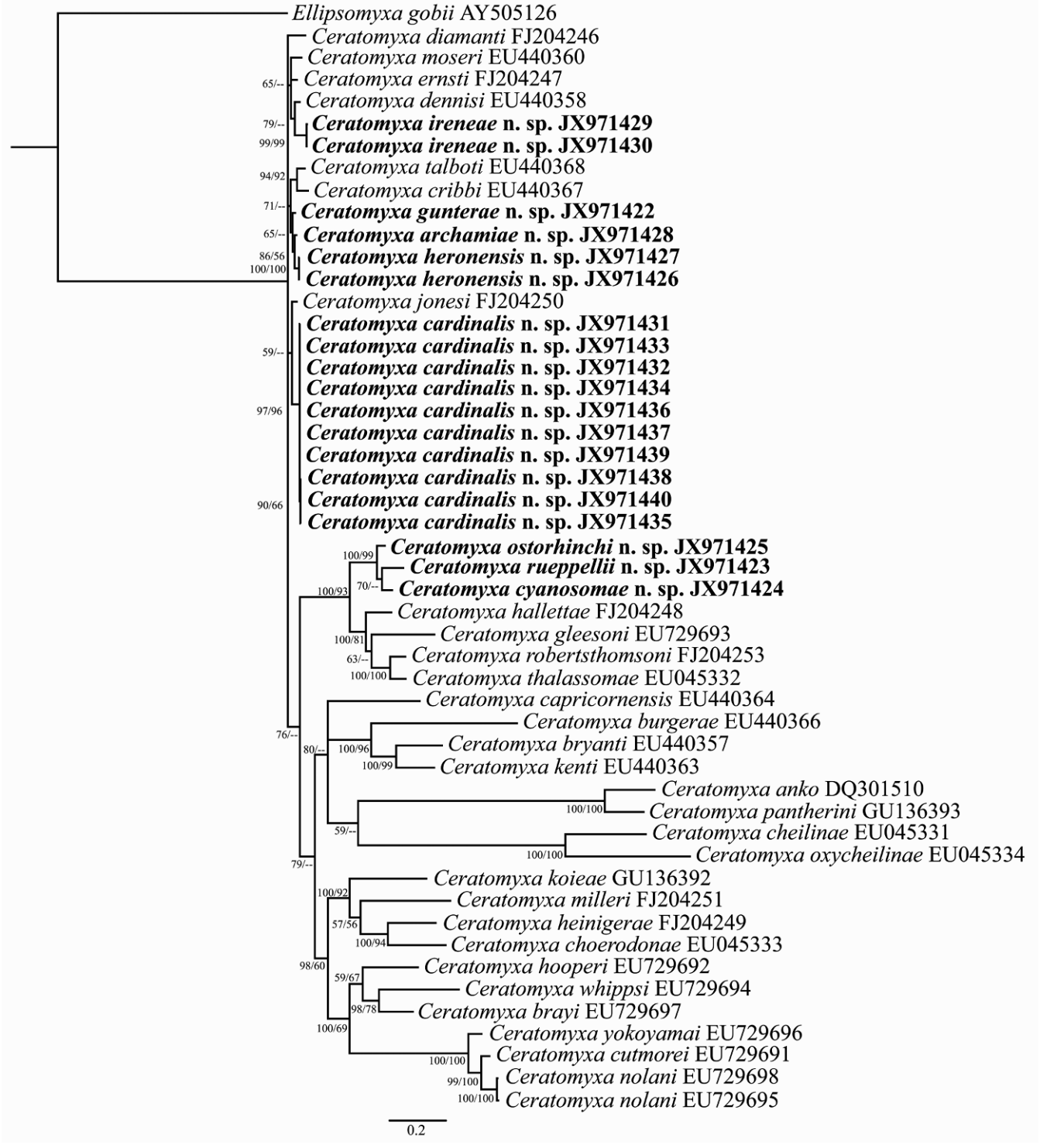

Fig. 5. Phylogenetic tree resulting from Bayesian analysis inferred from the SSU rDNA dataset. Support values at branching points are listed as: Posterior probabilities (PP) from Bayesian analysis/Bootstrap values from Maximum likelihood analysis. Values below 50% are indicated by dashes. Species from this study are shown in bold. GenBank accession number follows each taxon

cleotides (Table III). *Ceratomyxa cardinalis* sp. nov. is genetically most similar to *C. archamiae* sp. nov. and *C. jonesi* with 97% maximum sequence identity.

In addition, three LSU rDNA sequences of 1157–1162 bases were generated from three fish species (five isolates from 3 possible host/parasite/location combinations) for *C. cardinalis* sp. nov. Identical sequences (0% sequence variation) were generated from two isolates from *Nectamia fusca* from Heron Island (GenBank accession number: JX971442) and also two isolates from *Nectamia fusca* from Lizard Island (GenBank accession number: JX971443). The third sequence was generated from a single isolate taken from *Ostorhinchus aureus* from Ningaloo Reef (GenBank accession number: JX971441). There is 3–11 nucleotide differences (99–99.7% sequence identity) between the three LSU rDNA sequences generated for *C. cardinalis* sp. nov. (Table V). These sequences differ from the aligned LSU rDNA sequences of other *Ceratomyxa* species by 412–505 nucleotides (Table V). *Ceratomyxa cardinalis* sp. nov. is genetically most similar to *Ceratomyxa* sp. from *Gadus morhua* from the North Sea, off Scotland with which it shares 64.2% maximum sequence identity.

Principal Component Analyses (*PCA*)

Principal component analyses were performed to analyse the levels of both inter- and intra-specific morphological variation between and within the new species described above. PCA found little morphological distinction between the genetically distinct species, *C. gunterae* sp. nov., *C. rueppellii* sp. nov., *C. ostorhinchi* sp. nov. and *C. cyanosomae* sp. nov. (Fig. 4A). In contrast, the strong PCA separation of *C. archamiae* sp. nov. and *C. gunterae* sp. nov., the two species with the least inter-specific genetic variation, is consistent with the presence of two species (Fig. 4B). PCA comparison of *C. archamiae* sp. nov., *C. heronensis* sp. nov. and *C. ireneae* sp. nov., all species recorded from *A. fucata*, resulted in varying degrees of distinction (Fig. 4C). Minimal overlap was found between *C. archamiae* sp. nov. and *C. ireneae* sp. nov. (Fig. 4C). Although there is a greater morphological overlap between *C. ireneae* sp. nov. and *C. heronensis* sp. nov., subtle morphological variation is identified in this analysis (Fig. 4C). Significant overlap in morphology is observed for *C. heronensis* sp. nov. and *C. archamiae* sp. nov., although *C. heronensis* sp. nov. has a greater range in spore measurements than *C. archamiae* sp. nov. (Fig. 4C).

PCA found minimal intra-specific variation between isolates from two host species identified as *C. ireneae* sp. nov., with almost complete overlap of the two isolates, although the range for spore measurements from *Z. viridiventer* is greater than those from *A. fucata* (Fig. 4D). PCA of subsets of host/parasite/location combinations of isolates identified as *C. cardinalis* sp. nov. suggest a complex structure (Fig. 4E). Isolates comprising GenBank accession number JX971431 (which showed 0% intra-specific variation in sequence data) showed significant morphological variation (Fig. 4E). In particular, there was no overlap between isolates from *Cheilodipterus intermedius* from Heron Island (stars in Fig. 4E) and *Ostorhinchus cyanosoma* from Ningaloo Reef (circles in Fig. 4E). There was also minimal morphological overlap between the isolates from *O. cyanosoma* from Ningaloo Reef and *C. quinquelineatus* from Lizard Island or *Z. leptacantha* from Lizard Island (Fig. 4E). Similarly, minimal morphological overlap was found between isolates from *C. intermedius* from Heron Island (stars in Fig. 4E) and *C. artus* from Lizard Is-

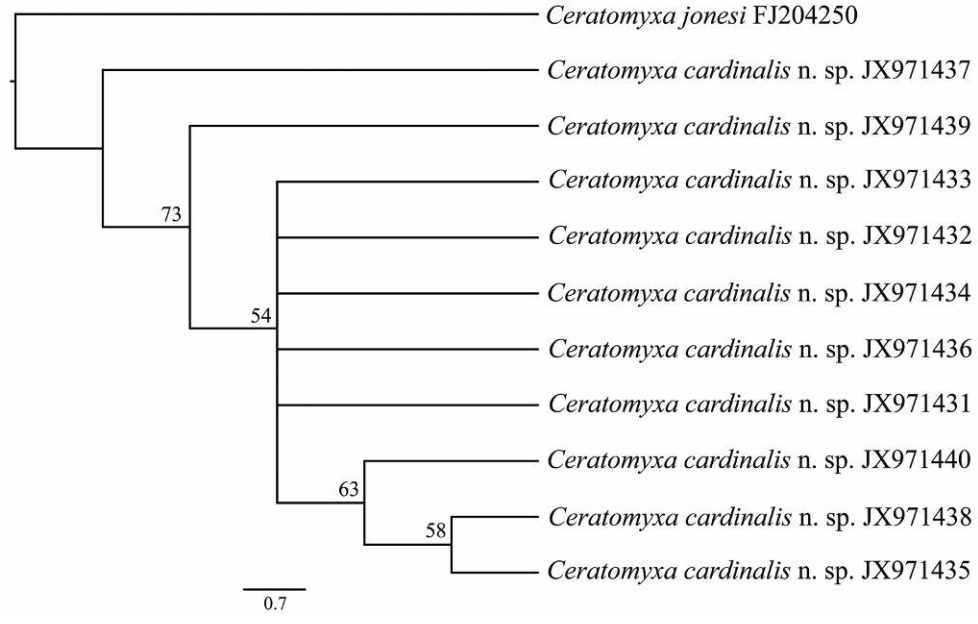

Fig. 6. Phylogenetic tree resulting from Neighbour-joining analysis inferred from the SSU rDNA dataset of all *Ceratomyxa cardinalis* sp. nov. sequences and *C. jonesi*. Bootstrap support values are shown at branching points. GenBank accession number follows each taxon

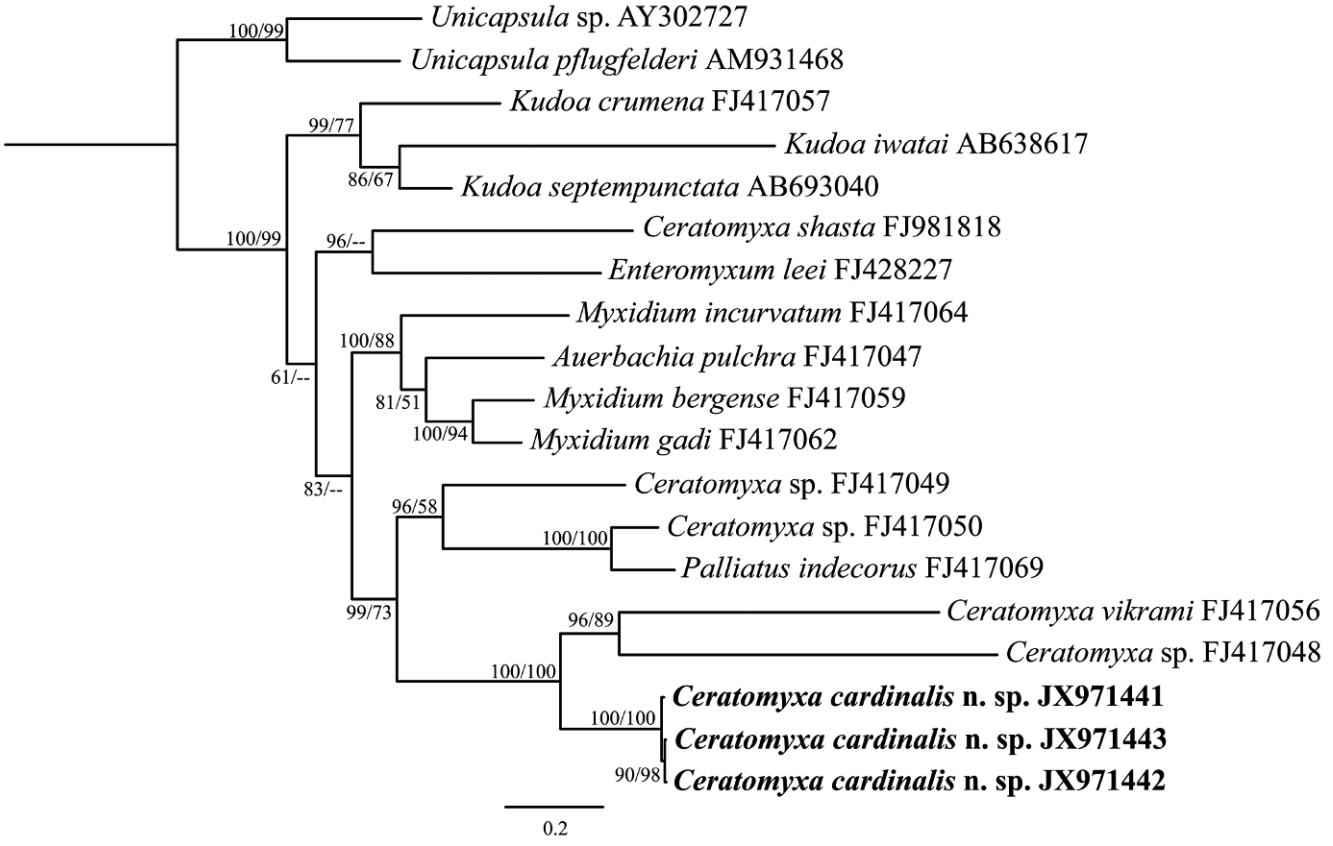

Fig. 7. Phylogenetic tree resulting from Bayesian analysis inferred from the LSU rDNA dataset. Support values at branching points are listed as: Posterior probabilities (PP) from Bayesian analysis/Bootstrap values from Maximum likelihood analysis. Values below 50% are indicated by dashes. Species from this study are shown in bold. GenBank accession number follows each taxon

land (crosses in Fig. 4E). However, when comparing all isolates of *C. cardinalis* sp. nov. (isolates represented in Fig. 4E are combined) there is minimal intra-specific morphological variation (Fig. 4F).

rDNA phylogenetic analyses

Molecular analyses included 31 SSU rDNA myxosporean sequences available from GenBank together with the sequences generated in this study (see Fig. 5). Bayesian inference and maximum likelihood analyses resulted in phylograms of similar topology but with varying levels of clade support (see Fig. 5). Relationships of the novel species of *Ceratomyxa* were largely unresolved, with the exception of *C. ostorhinchi* sp. nov., *C. cyanosomae* sp. nov. and *C. rueppellii* sp. nov. which formed a well-supported clade with *C. hallettae* Gunter, Whipps et Adlard, 2009, *C. thalassomae* Heiniger, Gunter et Adlard, 2008, *C. gleesoni* and *C. robertsthompsoni* Gunter, Whipps et Adlard, 2009. *Ceratomyxa gunterae* sp. nov., *C. archamiae* sp. nov. and all isolates of *C. heronensis* sp. nov. showed a close phylogenetic relationship to each other and formed a moderately supported clade with *C. talboti* and *C. cribbi*. Isolates of *C. ireneae* sp. nov. formed a moderately supported clade with *C. moseri* Gunter et Adlard, 2008, *C. ernsti* and *C. dennisi* Gunter et Adlard, 2008. All isolates of *C. cardinalis* sp. nov. formed a well-supported clade and showed a poorly supported phylogenetic relationship with *C. jonesi*. Neighbour-joining analysis of all isolates of *C. cardinalis* sp. nov. resulted in varying levels of resolution with poor to moderate bootstrap support and several relationships unresolved (see Fig. 6).

Due to the low specificity of primers used for LSU rDNA, only five sequences were generated, all isolates of *C. cardinalis* sp. nov. Molecular analyses included 16 LSU rDNA myxosporean sequences available from GenBank together with the sequences generated in this study (Fig. 7). Bayesian inference and maximum likelihood analyses resulted in phylograms of identical topology but with varying levels of clade support (see Fig. 7). All isolates of *C. cardinalis* sp. nov. formed a well-supported clade with all other *Ceratomyxa* species available, with the exception of *C. shasta. Palliatus indecorus* was also included in this clade.

Discussion

This study provides the first comprehensive investigation of *Ceratomyxa* species from apogonids and provides the first de-

scriptions of *Ceratomyxa* species in apogonids from Australian waters. The survey of 23 apogonid species revealed that 15 were infected with species of *Ceratomyxa*, with eight novel species confirmed through SSU rDNA analysis. Only a single species has been previously described from the Apogonidae, *Ceratomyxa apogoni* from *Ostorhinchus aureus* from waters off India (Narasimhamurti *et al*. 1990). *Ceratomyxa apogoni* was recently placed in *Ceratomyxa* after the review and subsequent synonymy of *Leptotheca* Thélohan, 1895, however the placement of this species within *Ceratomyxa*, and indeed any currently recognised genus, is equivocal due to the unusual spore shape (Gunter and Adlard 2010). *Ceratomyxa apogoni* is able to be distinguished from all the novel species presented here on the basis of its trapezium-shaped spores with transversely striated valves.

Recognition of species

Molecular systematics are now an invaluable and essential tool for the classification of myxosporeans. Traditional bivalvulidan classification based on spore morphology alone to distinguish between closely related species is difficult due to the paucity of informative characters at the light microscopic level and the plasticity of spores (Gunter *et al*. 2009; Heiniger *et al*. 2008; Sitja-Bobadilla and Alvarez-Pellitero 1993; Meglitsch 1960). Usually genetic variation is combined with morphological and/or biological differences to provide evidence for the recognition of novelty. However, several *Ceratomyxa* species presented here are cryptic in that they are biologically and morphologically indistinguishable. Molecular data (combined with biological differences) were critical for the discrimination of several morphologically similar species presented here.

Ceratomyxa gunterae sp. nov., *C. rueppellii* sp. nov., *C. ostorhinchi* sp. nov. and *C. cyanosomae* sp. nov. are morphologically similar with minimal variation in PCA analyses but are biologically (host and locality) and genetically distinct with 5.5–16.9% (77–222 nucleotides difference) inter-specific genetic variation. Similarly, *C. heronensis* sp. nov., *C. archamiae* sp. nov. and *C. ireneae* sp. nov. are morphologically and biologically (all three species are reported from *Archamia fucata*) similar but again all are genetically distinct with inter-specific genetic variation of 1.4–4.1% (18–54 nucleotides difference). Paradoxically, two morphologically distinct species *C. gunterae* sp. nov. and *C. archamiae* sp. nov. are genetically most similar with 1.2% (16 nucleotides difference) variation. *Ceratomyxa archamiae* sp. nov. and *C. heronensis* sp. nov. also show a close genetic relatedness with 1.4% inter-specific variation observed. The levels of inter-specific variation for species presented here is greater than those previously reported (1–1.3%) (Gunter and Adlard 2009; Gunter *et al*. 2009). There is no set percentage or number of nucleotides difference for discrimination of species, which clearly needs to be assessed for each individual isolate. The inter-specific genetic variation (and minimal intra-specific variation observed for both species, 0% for *C. archamiae* sp. nov. and 0–0.1% for *C. heronensis* sp. nov.) observed between *C. heronensis* sp. nov. and *C. archamiae* sp. nov. provide compelling evidence for the discrimination of these two species, despite their morphological and biological similarities (both recovered from *A. fucata* at Heron Island). It is clear that without genetic data, it would have been impossible to distinguish and establish these new species based solely on morphology and host data.

Multiple sequence replicates of SSU rDNA revealed no intra-specific variation for *C. gunterae* sp. nov. and *C. archamiae* sp. nov. However, 0–0.1% (0–1 nucleotide) difference was observed in *C. heronensis* sp. nov. collected from three individual hosts, 0–0.2% (0–3 nucleotides) difference was observed in *C. ireneae* sp. nov. collected from three host species (four individual hosts) and 0–0.5% (0–7 nucleotides) difference was observed in *C. cardinalis* sp. nov. collected from 11 host species (34 individual hosts) from three localities. With the exception of *C. cardinalis* sp. nov., the levels of intra-specific variation observed here are within those previously reported for species of *Ceratomyxa*. Gunter and Adlard (2009) reported levels of up to 0.15% in *C. nolani* and Gunter and Adlard (2008) reported levels of up to 0.36% (5 nucleotides difference) in *C. lunula*. *Ceratomyxa ireneae* sp. nov. and *C. cardinalis* sp. nov. are unusual in that unlike most *Ceratomyxa* species, they are reported from multiple host species. Isolates of *C. ireneae* sp. nov. show minimal genetic and morphological variation. However, biological differences combined with the observed morphological and molecular variation suggest that the genetic marker used in this study is unable to unambiguously determine the taxonomic identity of these isolates. Nevertheless, based on the current available data these isolates are conservatively assigned to *C. ireneae* sp. nov.

The isolates recovered here for *Ceratomyxa cardinalis* sp. nov. suggests a complexity that requires further analysis. Phylogenetic analyses suggest that samples from ten species of apogonids from Lizard Island, five from apogonids from Heron Island, and two from apogonids from Ningaloo Reef form a well-supported clade to the exclusion of all other recognised species. However, there is significant morphological, molecular and biological variation within this clade. SSU and LSU rDNA sequences differ between isolates by up to seven nucleotides for SSU and 11 nucleotides for LSU despite several replicates from individual host/locality combinations returning identical sequences. The use of host as an informative diagnostic character has become particularly valuable for morphologically and genetically closely related species of *Ceratomyxa* (see Alama-Bermejo *et al*. 2011, Gunter *et al.* 2010). However, in contrast to the majority of *Ceratomyxa* species described previously, *C. cardinalis* sp. nov. is reported here from an exceptional number (11 apogonid species) of host species.

There is no evident correlation between the genetic variation and host (both at the species and genus levels) or geographic locality. However, PCA analyses suggest that,

although there is significant overlap between the majority of isolates, several of the host/locality combinations of *C. cardinalis* sp. nov. (which are genetically identical) are recognisably distinct from each other morphologically. This observed morphological distinction combined with genetic identity cannot be ignored and presents two possibilities, both of which have been reported previously for myxosporeans. Either SSU rDNA is not a suitable genetic marker for the discrimination of closely related species (see Burger and Adlard 2010) or morphological characters are uninformative for taxonomic classification (see Heiniger *et al*. 2008).

In an attempt to assess the resolution of SSU rDNA and further assess the taxonomic status of closely related isolates, five individual LSU rDNA sequences from two host species and two localities were generated for *C. cardinalis* sp. nov. The LSU rDNA region was chosen due to the availability of comparative sequences of *Ceratomyxa* and because it has been shown to provide greater taxonomic resolution for closely related members of the Multivalvulida (see Burger and Adlard 2010). There was 0–0.3% (0–11 nucleotides difference) intraspecific genetic variation observed for *C. cardinalis* sp. nov. in LSU rDNA among the five sequences. Interestingly, in both instances where multiple isolates were available from the same host species taken from the same location there was 100% sequence identity, as was found in SSU rDNA. Although the nucleotide differences are greater in LSU rDNA than in SSU rDNA, the percentage difference is within the range previously reported for intra-specific variation of *Ceratomyxa* species within the SSU rDNA.

Unfortunately we were unable to generate LSU rDNA sequences from sufficient isolates to enable an assessment of the resolution of this genetic region for closely related isolates. For the present, we feel unable to distinguish between what can reasonably be interpreted as inter- and intra-specific variation (using both morphological and molecular characters). Therefore, on the basis of the above similarities and despite morphological, geographical and host differences we take a conservative approach and assign all isolates to *C. cardinalis* sp. nov. In doing so we predict that future evidence, e.g. more informative diagnostic characters and/or further isolates for comparison, will reveal the presence of multiple species for isolates of both *C. cardinalis* sp. nov. and *C. ireneae* sp. nov.

Host specificity

The recent advances in, and incorporation of, genetic information into species characterisation has made it possible to critically assess the host specificity of many *Ceratomyxa* species. Previous phylogenetic studies on host specificity have focused on *Ceratomyxa* species in the teleost families Labridae, Pomacentridae, Serranidae and Sparidae (see Gunter and Adlard 2008, 2009; Heiniger *et al*. 2008; Alama-Bermejo *et al*. 2011). These studies, along with new species characterisations incorporating genetic characters (Gunter *et al*. 2010; Gleeson and Adlard 2011), have demonstrated that species of *Ceratomyxa* are typically highly host specific, usually being restricted to a single host species. Only a single species has been found, as confirmed by SSU rDNA, in multiple hosts; *C. talboti* was reported from five pomacentrid species at Lizard Island, GBR, Australia (Gunter and Adlard 2008). An additional 34 *Ceratomyxa* species have been described from multiple hosts but these are characterised on the basis of spore morphology alone (see Gunter *et al*. 2009; Eiras 2006). Molecular evidence that only closely related hosts (i.e. within the same family) harbour the same *Ceratomyxa* species has led to the suggestion that species reported in hosts from multiple orders and families may represent species complexes (Gunter *et al*. 2009).

Six of the eight new *Ceratomyxa* species characterised here have, so far, been reported from only a single host species and support the strict host specificity previously reported. The exceptions are *C. ireneae* n. sp., reported from three apogonid species (*Zoramia viridiventer, Archamia fucata* and *A. zosterophora*) at Lizard Island and *C. cardinalis* n. sp., reported from 11 apogonid species (*Cheilodipterus intermedius*, *C. artus*, *C. quinquelineatus*, *Apogon doederleini*, *Nectamia fusca*, *Ostorhinchus aureus*, *O. cookii*, *O. cyanosoma*, *O. properuptus*, *O. rubrimacula* and *Zoramia leptacantha*) at Lizard and Heron Islands and Ningaloo Reef. Although these isolates are from the same host family, as discussed above, we predict the isolates currently assigned to *C. cardinalis* sp. nov. and *C. ireneae* sp. nov. are in fact species complexes. Therefore, until the taxonomic identity of the isolates that currently comprise these species is clarified, including them in any assessment of host specificity would be premature and misleading.

Species richness

Infections of *Ceratomyxa* were found in 65% (15/23) of apogonid species examined in this study. Although most apogonid species examined harboured only a single species of *Ceratomyxa*, *Apogon doederleini*, *Ostorhinchus aureus* and *O. cyanosoma* each harboured two and *Archamia fucata* harboured three. Between 1 and 10 individuals only were examined for each of the remaining 'uninfected' apogonid species. Thus, the observed absence of infection in these species is likely a reflection of low sample size rather than actual absence of parasites. A recent study by Gunter (2009) found that sample size had a profound effect on discovery of *Ceratomyxa* species, with the percentage of infection rising from 29.1% in species examined once to 87.5% in species that were examined at least 10 times. Here, if we consider only apogonid species that have been sampled at least 10 times, the percentage of infected species rises to 93% (13/14). It is clear from the above figures that *Ceratomyxa* is a species rich genus in teleosts from Australian waters.

The limitations of morphological characters for taxonomic identification of myxosporean species are now widely accepted. Molecular data are essential for current taxonomic classification and were crucial in the discrimination of mor-

phologically similar, cryptic species. However, morphological distinction cannot be ignored and our results suggest that SSU rDNA is not a sufficient genetic marker for the discrimination of all the closely related species presented here. Further research is needed to comprehensively assess LSU rDNA and other genetic markers as useful tools for the taxonomic discrimination of these species.

Acknowledgements. This study was funded by, and is a contribution from, the Australian node of the *CReefs* global research initiative (grant number: 209/29), a project generously sponsored by BHP Billiton in partnership with The Great Barrier Reef Foundation, the Australian Institute of Marine Science, the Australian Biological Resources Study and the Alfred P. Sloan Foundation. *CReefs* is a field program of the Census of Marine Life. We thank Dr Terrence Miller for technical advice, phylogenetic discussions and assistance in field collections, Jeff Johnson for assistance with fish identification, Dr Tom Cribb for discussion on species boundaries and assistance in field collections and Ricky Gleeson who assisted in field collection, and the staff at Heron Island and Lizard Island Research Stations for logistical support in the field.

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(Accepted May 17, 2013)