Re-establishment of the family Coccomyxidae and description of five novel species of *Auerbachia* and *Coccomyxa* (Myxosporea: Bivalvulida) parasites from Australian fishes

HOLLY HEINIGER[1,2], NICOLE L. GUNTER[1,2]† *and* ROBERT D. ADLARD[1,2]*

[1] *Biodiversity Program, Queensland Museum, PO Box 3300, South Brisbane, Queensland 4101, Australia*
[2] *School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland 4072, Australia*

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SUMMARY

Five novel species, *Auerbachia scomberoidi* n. sp., *Auerbachia chaetodoni* n. sp., *Auerbachia caranxi* n. sp., *Coccomyxa colurodontidis* n. sp. and *Coccomyxa gobiodoni* n. sp. are described from the gall bladders of marine teleosts. These species descriptions provide the first record of *Auerbachia* from Australian waters. Each species is characterized morphologically, including additional measurements for *Auerbachia* spp. and small subunit ribosomal DNA (SSU rDNA) sequences were determined for molecular phylogenetic analyses. All 5 species were each recovered from a single (and different) species of host. Phylogenetic analyses revealed a close genetic relatedness between members of *Auerbachia* and *Coccomyxa*. Based on these phylogenetic data, on obvious paraphyly displayed by the Myxidiidae and on priority, we propose the re-establishment of the family Coccomyxidae to house all species of the genera, *Coccomyxa*, *Auerbachia* and *Globospora*.

Key words: Myxosporea, Bivalvulida, *Auerbachia*, *Coccomyxa*, Coccomyxidae, gall bladder, parasite, phylogeny.

INTRODUCTION

Myxosporean research in Australia has made a significant contribution to the discovery of bivalvulidan species, with a total of 72 new species descriptions, 26 new host records (Delvinquier, 1986; Hill *et al*. 1997; Rothwell *et al*. 1997), and reports of a further 58 undescribed species (O'Donoghue and Adlard, 2000; Roubal, 1994*a*, *b*). These records include 44 species of *Ceratomyxa* (Johnston and Bancroft, 1918; Moser *et al*. 1989; Su and White, 1994; Heiniger *et al*. 2008; Gunter and Adlard, 2008, 2009; Gunter *et al*. 2009, 2010), 10 *Myxobolus* species (Johnston and Bancroft, 1918; Langdon, 1990; Lom and Dykova, 1994; Su and White, 1994) and several species of *Sphaeromyxa* (Su and White, 1994; Lom, 2004), *Myxidium* (see Johnston and Bancroft, 1918; Gunter and Adlard, 2008), *Zschokkella* (Moser *et al*. 1989; Su and White, 1994, 1995), *Ortholinea* (Kent and Moser, 1990; Lom *et al*. 1992; Su and White, 1994), *Triangula* (Langdon, 1987), *Coccomyxa* (Lom *et al*. 1992), *Sinuolinea* (Moser *et al*. 1989), *Sphaerospora* (Moser *et al*. 1989; Su and White, 1994), *Chloromyxum* (Woolcock, 1936) and *Henneguya* (Hallet and Diamant, 2001; Johnston and Bancroft, 1918). The rate of description of new species from Australian waters has not slowed in recent years. This study reports 2 new *Coccomyxa* and 3 new *Auerbachia* species representing the first descriptions from the latter genus in Australian waters.

Since the establishment of *Coccomyxa* Léger & Hesse 1907 and *Auerbachia* Meglitsch 1968 few species have been recorded and described and thus little is known beyond descriptions derived from light microscope observations. To date, there are 7 species assigned to the genus *Auerbachia*, all united in having club-like spores with a broad anterior end, narrow caudal extension and a single elongated polar capsule opening at the anterior end of the spore (Lom and Dykova, 2006). Genus *Coccomyxa* has 9 species assigned, all with spores that are ellipsoidal with a single elongated polar capsule opening in the sutural plane (Lom and Dykova, 2006) with the exception of *Coccomyxa leognathi* which is club shaped (Wu, 1991). Species of *Auerbachia* and *Coccomyxa* are mainly coelozoic in marine teleosts with the exception of 2 species reported as histozoic in their hosts, *Auerbachia hepatica* infecting the liver of *Carangoides praeustus* (Sarkar, 2006) and *Coccomyxa hoffmani* infecting the gill cartilage of *Plotosus anguillaris* (Cheung and Nigrelli, 1990). Pathological changes have been reported for only one species, *Coccomyxa jirilomi*, which caused cholestasis, periductular fibrosis and pericholangitis in *Bathygobius cyclopterus* (Diamant *et al*. 2007). Three species have been

* Corresponding author: Biodiversity Program, Queensland Museum, PO Box 3300, South Brisbane, Queensland 4101, Australia. Tel: +617 3840 7723. Fax: +617 3846 1226. E-mail: robert.adlard@qm.qld.gov.au
† Current Address: Australian National Insect Collection, Commonwealth Scientific and Industrial Research Organisation Ecosystem Sciences, Black Mountain Laboratories, PO Box 1700, Canberra, ACT, 2601 Australia.

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genetically characterized using SSU rDNA: *Auerbachia pulchra* in *Coryphaenoides rupestris* from the Atlantic Ocean (Fiala, 2006), *C. jirilomi* in *Bathygobius cyclopterus* from the Red Sea and *Coccomyxa* sp. IE200903 in *Istiblennius edentulous* also from the Red Sea (Diamant *et al*. 2007). Herein we propose 3 novel species of *Auerbachia* and 2 novel species of *Coccomyxa*. We further propose to re-establish the Coccomyxidae and to transfer all *Coccomyxa* spp. and the genera and species currently within the Auerbachiidae into the Coccomyxidae.

MATERIALS AND METHODS

Host and parasite collection

Teleost fish were collected by line and spear fishing from Heron Island (23°26′S, 151°54′E) at the southern end of the Great Barrier Reef (GBR) and Lizard Island at the northern end of the GBR (14°40′ S, 145°27′E), Queensland, Australia and at Point Cloates on Ningaloo Reef (22°40′S, 113°41′E), off Western Australia. Fish were euthanized by neural pithing. The gall bladder was removed from the abdominal cavity and ruptured in an excavated glass block. A small drop of bile was placed on a glass microscope slide, covered with a glass cover-slip and examined with a light microscope at 400x magnification. Infected gall bladders were preserved in 100% ethanol for DNA analysis and frozen in saline for morphological characterization.

Morphological analysis of spores

Measurements of spores followed those described for the type species of each genus (Lèger and Hesse, 1907; Meglitsch, 1968) with the additional measurement of caudal extension angle for *Auerbachia* isolates (Fig. 1). The caudal extension angle was made from images where the spore presented a maximum caudal flexion. Two axes were then constructed, one bisecting the main body of the spore and another bisecting the posterior caudal extension, which when extended, meet at the position where the posterior angle is measured (see Fig. 1). Images of 30 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 characterization of each isolate. All measurements are given in micrometers (μm).

Principal component analysis of the morphometric data was conducted using PAST v 1.97 (Hammer *et al*. 2001) and 95% ellipses from individual scatter plots of components 1 and 2 were obtained

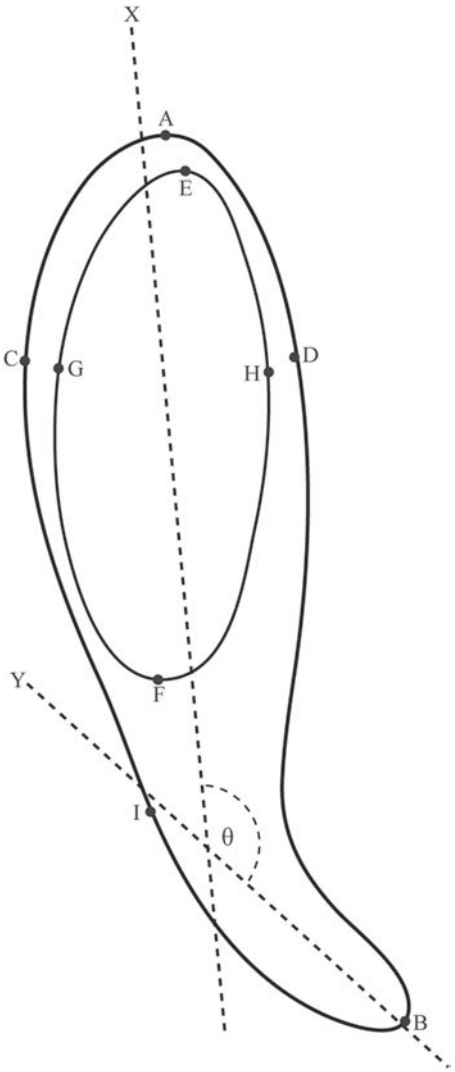

Fig. 1. Schematic diagram showing spore measurements for *Auerbachia*. A-B, Spore length; C-D, spore width; E-F, polar capsule length; G-H, polar capsule width; I-B, caudal extension length; X, axis bisecting the body of the spore; Y, axis bisecting the posterior caudal extension; θ, caudal extension angle.

of variant-covariant matrices from *Auerbachia* and *Coccomyxa* measurements. Discriminant analysis was also conducted on morphometric data of *Auerbachia* and *Coccomyxa* species in order to determine which morphological character contributed the most to observed variation between groups or species using Systat 11 software (Systat Software Inc. Chicago, IL, USA).

Small subunit 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 min and the ethanol supernatant removed. DNA was extracted from the pellet as per the recommended protocol accompanying the QIAgen DNeasy Kit (QIAGEN Inc., Valencia, CA, USA). Small subunit ribosomal DNA (SSU rDNA) was amplified by PCR using the primers MyxospecF

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5′ TTC TGC CGT ATC AAC TWG TTG (Fiala, 2006) and 18R 5′ CTA CGG AAA CCT TGT TAC G (Whipps *et al*. 2003). PCR reactions and purification were performed as described by Heiniger *et al*. (2008). Purified DNA was sent to the Australian Genome Research Facility, The University of Queensland, Australia, for sequence determination using the same primers as used for the initial amplification.

Phylogenetic analysis

The SSU rDNA regions from the taxa sequenced in this study were edited using BioEdit version 7.0.9 (Hall, 1999). Selected SSU rDNA sequences were downloaded from GenBank and included all described *Auerbachia* and *Coccomyxa* sequences available together with all sequences from a recent myxosporean phylogeny (Diamant *et al*. 2007) and some Australian myxosporean species that were returned in BLAST results. All new sequences generated in this study were lodged in GenBank. An alignment of all the taxa included here was produced using Muscle version 3.7 (Edgar, 2004) using the Clustal W algorithm (Thompson *et al*. 1994) with UPGMB parameters for all iterations on the CIPRES portal (Miller *et al*. 2009). The resulting alignment was exported as fasta and nexus files, edited by eye and trimmed using MacClade version 4.08 (Maddison and Maddison, 2005). This produced a 1935 base alignment that was used to conduct all phylogenetic analyses. A second alignment including the species described here and other species of *Auerbachia* and *Coccomyxa* was created in BioEdit version 7.0.9 (Hall, 1999) using sequence alignment parameters as recommended by Hall (2001). The resulting 1252 base alignment was used to produce a distance matrix to view nucleotide base differences.

Neighbour-joining and parsimony analyses were conducted using PAUP* 4.0b 10 (Swofford, 2002) and Bayesian analysis using MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003). Neighbour-joining and parsimony analyses were performed using default parameters to construct the trees. The strength of resultant relationships was tested by bootstrap analyses with 10 000 replicates. Parsimony analysis employed a heuristic search with 50 repetitions of random sequence addition and tree bisection and reconnection branch swapping. The software jModelTest version 0.1.1 (Posada, 2008) was used to estimate the best substitution model for the SSU rDNA dataset. Bayesian analysis was conducted using the GTR + I + G model predicted as the best estimator by the Akaike Information Criterion (AIC) in jModelTest. Bayesian inference analysis was 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, ngammacat = 4, the MCMC parameters were left at the default settings, and the priors parameters of the combined dataset were set to ratepr = fixed as per the model selected by jModelTest for AIC. Samples of substitution model parameters, and tree and branch lengths were summarized 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.

RESULTS

Since 2005, over 2200 individual teleosts and elasmobranchs from 70 families have been examined for the presence of myxosporean infections at 10 different localities off the Great Barrier Reef, southern coast of Queensland and Ningaloo Reef in Western Australia (Adlard, unpublished data). Fish sampling has been both opportunistic and targeted. However, despite this extensive survey only 7 isolates (6 possible host/parasite combinations) of *Auerbachia* (including the 3 species reported here) from 2 locations in Queensland and from 3 host families (Carangidae, Chaetodontidae and Polynemidae) and only the 2 species of *Coccomyxa* reported here, have been discovered.

Phylum Myxozoa
Order Myxosporea
Class Bivalvulida
Family Coccomyxidae Léger & Hesse, 1907
Syn: Auerbachiidae Evdokimova, 1973
Genus *Auerbachia* Meglitsch, 1968
***Auerbachia scomberoidi* n. sp.**
(Figs 2 and 3, Tables 1 and 3)

Description: Spores typical of Genus *Auerbachia*. Mature spores club-like with broad anterior section and narrow caudal extension, 21·4 ± 1·91 (17·5–25·2) in total length and 7·5 ± 0·76 (6·3–10) in width. Caudal extension 6·6 ± 1·47 (4·4–9·6) in length and at an angle of 143·7° ± 14·86 (98–176) to the spore. Shell valves smooth and suture indistinct. Single elongate pyriform polar capsule situated at the anterior end of the spore, 10·6 ± 0·69 (9·3–12) in length and 3·8 ± 0·39 (3–4·5) in width. Polar filament with 2 longitudinal coils.
Material: Syntypes – air-dried slide stained with Giemsa, number G465434 and DNA voucher, number G465435 deposited in the collections of the Queensland Museum, Brisbane, Australia. GenBank Accession number HM037787.
Host: Scomberoides lysan (Forsskål, 1775) (Double-spotted Queenfish), Family Carangidae.
Prevalence: 1 of 2 (50%)
Locality: Lizard Island, Great Barrier Reef, Queensland (14°40′S, 145°27′E).
Site: Gall bladder

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Etymology: Named after the host genus, *Scomberoides,* used as a substantive in the genitive case.
Taxonomic affinities: Auerbachia scomberoidi n. sp. is superficially similar in size to *A. anomala, A. monstrosa* and *Auerbachia* sp. of Yoshino and Noble (1973) (see Table 1). *Auerbachia scomberoidi* n. sp. can be distinguished from these 3 species by having a smaller caudal extension length and narrower spore width. Measurements of *Auerbachia* sp. were taken from ethanol preserved material and are therefore compromised for comparative purposes since preservation has been shown to cause spores to shrink (Parker and Warner, 1970).
Remarks: A total of 1321 bases of SSU rDNA were generated from *A. scomberoidi* n. sp. (GenBank Accession number HM037787). The sequence differs from the aligned sequences of *Auerbachia* spp. and *Coccomyxa* spp. at 15–74 of 1252 nucleotides and has a maximum genetic similarity of 98·77% with *A. caranxi* n. sp. (Table 3).

Auerbachia chaetodoni n. sp. (Figs 2 and 3, Tables 1 and 3)

Description: Spores typical of Genus *Auerbachia*. Mature spores club-like with broad anterior section and narrow caudal extension, 32·2 ± 2·36 (26·9–37·7) in total length and 9·1 ± 0·57 (8–10·1) in width. Caudal extension is 12·2 ± 2·16 (7·6–17·3) in length and at an angle of 142·6° ± 18·36 (107·9–169·5) to the spore. Shell valves smooth and suture indistinct. Single elongate pyriform polar capsule situated at the anterior end of the spore, 15·8 ± 1·09 (13–18·2) in length and 6·1 ± 0·44 (5·4–6·9) in width. Polar filament with 5 coils.
Material: Syntypes – air-dried slides stained with Giemsa, numbers G465436 and G465437. Voucher specimens, air-dried slide stained with Giemsa, number G465438 and DNA voucher, number G465439 deposited in the collections of the Queensland Museum, Brisbane, Australia. GenBank Accession number HM037788.
Host: Chaetodon unimaculatus, Bloch, 1787 (Teardrop Butterflyfish), Family Chaetodontidae.
Prevalence: 1 of 6 (16·67%)
Locality: Lizard Island, Great Barrier Reef, Queensland (14°40′S, 145°27′E).
Site: Gall bladder
Etymology: Named after the host genus, *Chaetodon,* used as a substantive in the genitive case.
Taxonomic affinities: Auerbachia chaetodoni n. sp. is superficially similar in size to *A. hepatica* and *A. pulchra* (see Table 1). *Auerbachia chaetodoni* n. sp. differs from these two species by having a narrower spore. *Auerbachia hepatica* has a larger polar capsule and a longer caudal extension and *A. pulchra* has a smaller polar capsule. *Auerbachia hepatica* also differs from all other described *Auerbachia* species in its host tissue location (histozoic in the liver). Furthermore, measurements of *A. hepatica* are unreliable for comparative purposes as they were derived from formalin-preserved material that has been shown to cause spores to shrink (Parker and Warner, 1970).
Remarks: A total of 1305 bases of SSU rDNA were generated from *A. chaetodoni* n. sp. (GenBank Accession number HM037788). The sequence differs from the aligned sequences of *Auerbachia* spp. and *Coccomyxa* spp. at 21–85 of 1252 nucleotides and has a maximum genetic similarity of 98·28% with *A. caranxi* n. sp. (Table 3).

Auerbachia caranxi n. sp. (Figs 2 and 3, Tables 1 and 3)

Description: Spores typical of Genus *Auerbachia*. Mature spores club-like with broad anterior section and narrow caudal extension, 16·8 ± 1·01 (14·2–18·8) in total length and 6·7 ± 0·4 (6–7·9) in width. Caudal extension is 6·2 ± 0·84 (4·9–7·9) in length and at an angle of 157·5° ± 9·44 (123·5–172·2) to the spore. Shell valves smooth and suture indistinct. Single elongate pyriform polar capsule situated at the anterior end of the spore, 8·3 ± 0·8 (7–9·7) in length and 3·2 ± 0·34 (2·4–3·8) in width. Polar filament with 4 coils.
Material: Syntypes – air-dried slides stained with Giemsa, numbers G465440 and G465441. Voucher specimens, air-dried slide stained with Giemsa, number G465442 and DNA voucher, number G465443 deposited in the collections of the Queensland Museum, Brisbane, Australia. GenBank Accession number HM037789.
Host: Caranx papuensis, Alleyne & MacLeay, 1877 (Brassy Trevally), Family Carangidae
Prevalence: 2 of 6 (33·33%)
Locality: Lizard Island, Great Barrier Reef, Queensland (14°40′S, 145°27′E).
Site: Gall bladder
Etymology: Named after the host genus, *Caranx,* used as a substantive in the genitive case.
Taxonomic affinities: Auerbachia caranxi n. sp. is superficially similar in size to *Auerbachia chorinemusi* (see Table 1). *Auerbachia caranxi* n. sp. has a slightly narrower spore, shorter polar capsule and longer caudal extension than *A. chorinemusi*.
Remarks: A total of 1312 bases of SSU rDNA was generated from *A. caranxi* n. sp. (GenBank Accession number HM037789). The sequence differs from the aligned sequences of *Auerbachia* spp. and *Coccomyxa* spp. at 15–76 of 1252 nucleotides and has a maximum genetic similarity of 98·77% with *A. scomberoidi* n. sp. (Table 3).

Genus *Coccomyxa* Léger and Hesse, 1907

Coccomyxa colurodontidis n. sp. (Figs 2 and 3, Tables 2 and 3)

Description: Spores typical of the Genus *Coccomyxa*. Mature spores ellipsoidal, 12·1 ± 0·9 (10·2–14·2) in

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Table 1. Mean spore dimensions including range in μm and caudal extension angle in degrees, for all available *Auerbachia* spp.

(PC: polar capsule; CE: caudal extension.)

Species	Host	Locality	Total length	Spore width	PC Length	PC Width	CE Length	CE Angle
***A. scomberoidi* n. sp.**	***Scomberoides lysan***	**GBR, Australia**	**21·4 (17·5–25·2)**	**7·5 (6·3–10)**	**10·6 (9·3–12)**	**3·8 (3–4·5)**	**6·6 (4·4–9·6)**	**143·7 (98–176)**
***A. chaetodoni* n. sp.**	***Chaetodon unimaculatus***	**GBR, Australia**	**32·2 (26·9–37·7)**	**9·1 (8–10·1)**	**15·8 (13–18·2)**	**6·1 (5·4–6·9)**	**12·2 (7·6–17·3)**	**142·6 (107·9–169·5)**
***A. caranxi* n. sp.**	***Caranx papuensis***	**GBR, Australia**	**16·8 (14·2–18·8)**	**6·7 (6–7·9)**	**8·3 (7–9·7)**	**3·2 (2·4–3·8)**	**6·2 (4·9–7·9)**	**157·5 (123·5–172·2)**
Auerbachia anomala Meglitsch, 1968	*Genypterus blacodes*	New Zealand	22·4 (20·7–24·3)	8·8 (7·8–9)	9·5 (8·4–10·7)	3·1 (2·8–3·4)	10·1 (9–10·7)	
Auerbachia chorinemusi Padma-Dorothy, Kalavati & Vaidchi, 1998	*Scomberoides tol* (= *Chorinemus tol*)	Bay of Bengal, India	16·2 (15·48–18·06)	7·19 (6·02–9·46)	8·64 (7·74–9·46)	3·82 (3·44–5·16)	3·89 (3·44–5·16)	
Auerbachia hepatica Sarkar, 2006	*Carangoides praeustus*	Bay of Bengal, India	33·21 (31·45–37·4)	15·38 (13·6–17·85)	15·92 (13·6–18·7)	5·14 (4·25–5·95)	10·77 (9·35–11·9)	
Auerbachia monstrosa Meglitsch, 1968	*Coelorhynchus australis*	New Zealand	25·2 (21·1–28·9)	9·5 (9·3–10·3)	11·2 (9·8–13·2)	4·3 (3·9–4·9)	12·2 (9·8–15·7)	
Auerbachia pulchra Lom, Noble & Laird, 1975	*Macrourus berglax*	Newfoundland and Iceland	30 (26–34)	11 (11–12)	12 (9–14)	4 (3·5–5)		
Auerbachia bajadi Abdel-Baki, 2010	*Carangoides bajad*	Red Sea, Egypt	20 (19–21)	8 (7–9)	9 (8–10)	4 (3–5)	10 (9–11)	
Auerbachia sp. of Yoshino & Noble	*Macrourus berglax*	Newfoundland and Iceland	22·96 (17–28)	8·03 (7–10)	10·17 (7·5–13·5)	3·91 (3–5·5)	9·67 (6–13)	

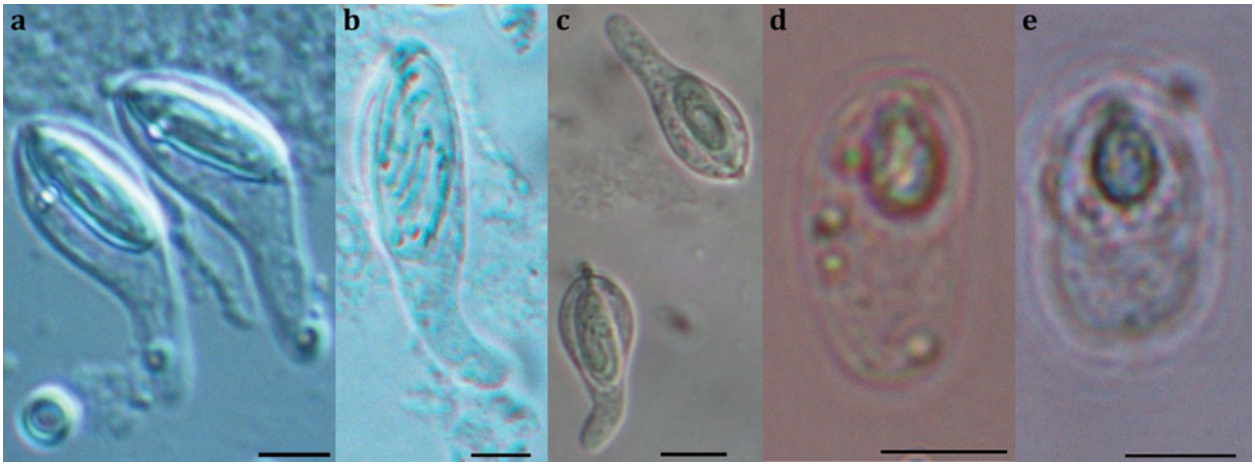

Fig. 2. Photomicrographs of spores of new *Auerbachia* and *Coccomyxa* species. (a) *A. scomberoidi* n. sp.; (b) *A. chaetodoni* n. sp.; (c) *A. caranxi* n. sp.; (d) *C. colurodontidis* n. sp.; (e) *C. gobiodoni* n. sp. Scale bar = 5 μm.

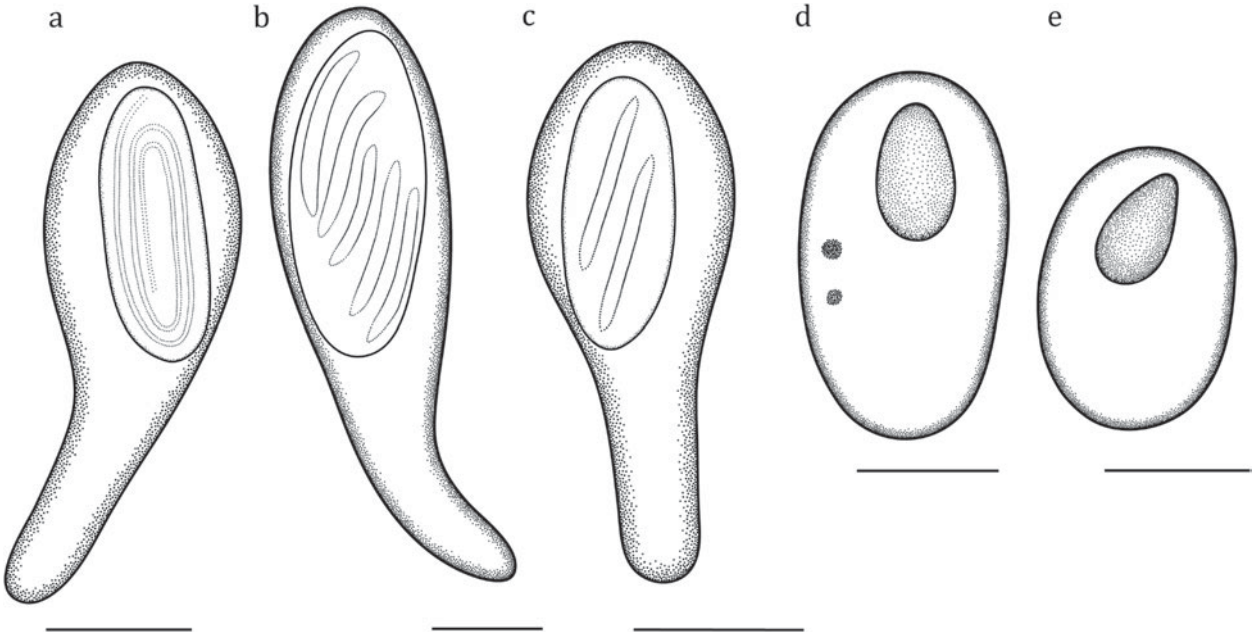

Fig. 3. Diagrammatic illustrations of spores of the new *Auerbachia* spp. and *Coccomyxa* spp. (a) *A. scomberoidi* n. sp.; (b) *A. chaetodoni* n. sp.; (c) *A. caranxi* n. sp.; (d) *C. colurodontidis* n. sp.; (e) *C. gobiodoni* n. sp. Scale bar = 5 μm.

length and 6 ± 0·5 (5·2–7·1) in width. Shell valves smooth and suture indistinct. Single pyriform polar capsule situated at one pole of the spore, 4·9 ± 0·5 (3·8–5·8) in length and 2·7 ± 0·33 (2·1–3·5) in width. Polar filament indistinct.

Material: Syntypes – air-dried slide stained with Giemsa, number Z27552 deposited in the collections of the Western Australian Museum, Perth, Australia. Voucher specimens, air-dried slides stained with Giemsa, numbers G465444–G465445 and DNA Voucher, number G465446 in the collections of the Queensland Museum, Brisbane, Australia. GenBank Accession number HM037790.

Host: Colurodontis paxmani, Hutchins, 1977 (Paxman's Leatherjacket), Family Monacanthidae

Prevalence: 3 of 4 (75%)

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

Site: Gall bladder

Etymology: Named after the host genus, *Colurodontis,* used as a substantive in the genitive case.

Taxonomic affinities: Coccomyxa colurodontidis n. sp. is superficially similar in size to *C. baleswarensis, C. jirilomi, C. meridiei, C. tenuiparies, C. hoffmani, C. ovale* and *C. morovi* (see Table 2). *Coccomyxa colurodontidis* n. sp. can be distinguished from *C. baleswarensis* by its larger spore and smaller polar capsule. *Coccomyxa colurodontidis* n. sp. has a longer spore than *C. jirilomi* and *C. meridiei, C. tenuiparies* has a wider spore and longer polar capsule.

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Table 2. Mean spore dimensions including range in μm for species of *Coccomyxa* with superficial morphological similarity to *Coccomyxa colurodontidis* n. sp. and *Coccomyxa gobiodoni* n. sp.

(PC: polar capsule.)

Species	Host	Locality	Total length	Spore width	PC Length	PC Width
***C. colurodontidis* n. sp.**	***Colurodontis paxmani***	**Ningaloo Reef, Australia**	**12·1 (10·2–14·3)**	**6 (5·2–7·1)**	**4·9 (3·8–5·8)**	**2·7 (2·1–3·5)**
***C. gobiodoni* n. sp.**	***Gobiodon citrinus***	**GBR, Australia**	**10·6 (8·4–12·4)**	**6·5 (5·5–7·5)**	**3·8 (2·9–4·7)**	**2·5 (1·9–3·2)**
Coccomyxa baleswarensis Sarkar, 1995	*Tenualosa ilisha* (=*Hilsa ilisha*)	Bay of Bengal, India	11·36 (10–13)	5·17 (4·5–6)	5·2 (4–6)	2·43 (2–3)
Coccomyxa jirilomi Diamant, Lipshitz & Ucko, 2007	*Bathygobius cyclopterus*	Red Sea, Israel	10·1 (9–11·3)	6·1 (5–7)	5·1 (3·5–5·7)	2·7 (1·9–3·2)
Coccomyxa meridiei Lom, Rhode & Dykova, 1992	*Herklosichthys castelnaui*	NSW, Australia	10·4 (9·2–11·8)	6·2 (5·3–7·8)	4·3 (3·1–5·8)	2·7 (2·3–3·1)
Coccomyxa tenuiparies Lom, Rhode & Dykova, 1992	*Heteroclimus whiteleggii*	NSW, Australia	11 (8·8–12·5)	9 (7·3–10·8)	5·6 (4·7–7·3)	2·9 (2·6–3·1)
Coccomyxa hoffmani Cheung & Nigrelli, 1990	*Plotosus anguillaris*	Indo-Pacific, Philippines	7·5–9·5	6–7·5	5–8	2·5–3·5
Coccomyxa ovale Kovaljova & Gajevskaja, 1968	*Beryx splendens*	North Atlantic	10·6–12	4·8–6·7	3·5–5·2	2·7
Coccomyxa morovi Leger & Hesse, 1907	*Sardina pilchardus* (=*Clupea pilchardus*)	Mediterranian Sea	14	5–6	6	
Coccomyxa sp. of Diamant, Lipshitz & Ucko	*Istiblennius edentulus*	Red sea, Israel	7·6–9·6	4·2–5·2	3·5	2·4

Coccomyxa hoffmani is distinguished from *C. colurodontidis* n. sp. by its site of infection (gill cartilage), shorter spore and longer polar capsule. *Coccomyxa ovale* also has a shorter spore than *C. colurodontidis* n. sp., while *C. morovi* has a longer polar capsule.

Remarks: Two identical sequences were generated from individual hosts and aligned to create a total of 1350 bases of SSU rDNA from *C. colurodontidis* n. sp. (GenBank Accession number HM037790). This sequence differs from the aligned sequences of all other *Coccomyxa* spp. and *Auerbachia* spp. at 25–82 of 1252 nucleotides and has a maximum genetic similarity of 97·95% with *C. jirilomi* (Table 3).

Coccomyxa gobiodoni n. sp. (Figs 2 and 3, Tables 2 and 3)

Description: Spores typical of the Genus *Coccomyxa*. Mature spores ellipsoidal, 10·6 ± 1·03 (8·4–12·4) in length and 6·5 ± 0·57 (5·5–7·5) in width. Shell valves smooth and suture indistinct. Single pyriform polar capsule situated at one pole of the spore, 3·8 ± 0·41 (2·9–4·7) in length and 2·5 ± 0·25 (1·9–3·2) in width. Polar filament indistinct.

Material: Syntypes – air-dried slides stained with Giemsa, number G465447 and DNA Voucher number G465448 deposited in the collections of the Queensland Museum, Brisbane, Australia. GenBank Accession number HM037791.

Host: Gobiodon citrinus, (Rüppell, 1838) (Poison Goby), Family Gobiidae

Prevalence: 1 of 3 (33·3%)

Locality: Heron Island, Great Barrier Reef, Queensland (23°26′S, 151°54′E).

Site: Gall bladder

Etymology: Named after the host genus, *Gobiodon*, used as a substantive in the genitive case.

Taxonomic affinities: Coccomyxa gobiodoni n. sp. is superficially similar in size to *C. baleswarensis, C. jirilomi, C. meridiei, C. tenuiparies, C. hoffmani, C. ovale* and *C. morovi* (see Table 2). *Coccomyxa gobiodoni* n. sp. can be distinguished from *C. baleswarensis, C. jirilomi, C. meridiei, C. tenuiparies, C. hoffmani* and *C. morovi* by its shorter polar capsule. *Coccomyxa gobiodoni* n. sp. can be further distinguished from *C. baleswarensis* and *C. tenuiparies* by its difference in spore width. *Coccomyxa hoffmani* has a shorter spore than *C. gobiodoni* n. sp., while *C. morovi* has a longer spore. *Coccomyxa ovale* is able to be distinguished from *C. gobiodoni* n. sp. by its narrower spore.

Remarks: A total of 1337 bases of SSU rDNA was generated from *C. gobiodoni* n. sp. (GenBank Accession number HM037791). The sequence differs from the aligned sequences of all other species of *Coccomyxa* spp. and *Auerbachia* spp. at 22–85 of 1252 nucleotides and has a maximum genetic similarity of 98·20% with *C. jirilomi* (Table 3).

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Table 3. Similarity in rDNA sequences of all *Auerbachia* and *Coccomyxa* species

(Percentage of nucleotide differences shown above the oblique and actual nucleotide differences shown below the oblique.)

	1	2	3	4	5	6	7	8
1. *C. gobiodoni* n. sp.		97·70	98·20	96·61	92·75	95·65	96·34	96·00
2. *C. colurodontidis* n. sp.	28		97·95	97·20	93·02	95·57	96·51	96·34
3. *C. jirilomi*	22	25		96·86	92·84	95·31	96·34	95·74
4. *Coccomyxa* sp.	41	34	38		92·30	95·49	95·92	95·92
5. *A. pulchra*	85	82	84	90		92·70	93·70	93·52
6. *A. chaetodoni* n. sp.	52	53	56	54	85		98·03	98·28
7. *A. scomberoidi* n. sp.	44	42	44	49	74	24		98·77
8. *A. caranxi* n. sp.	48	44	51	49	76	21	15	

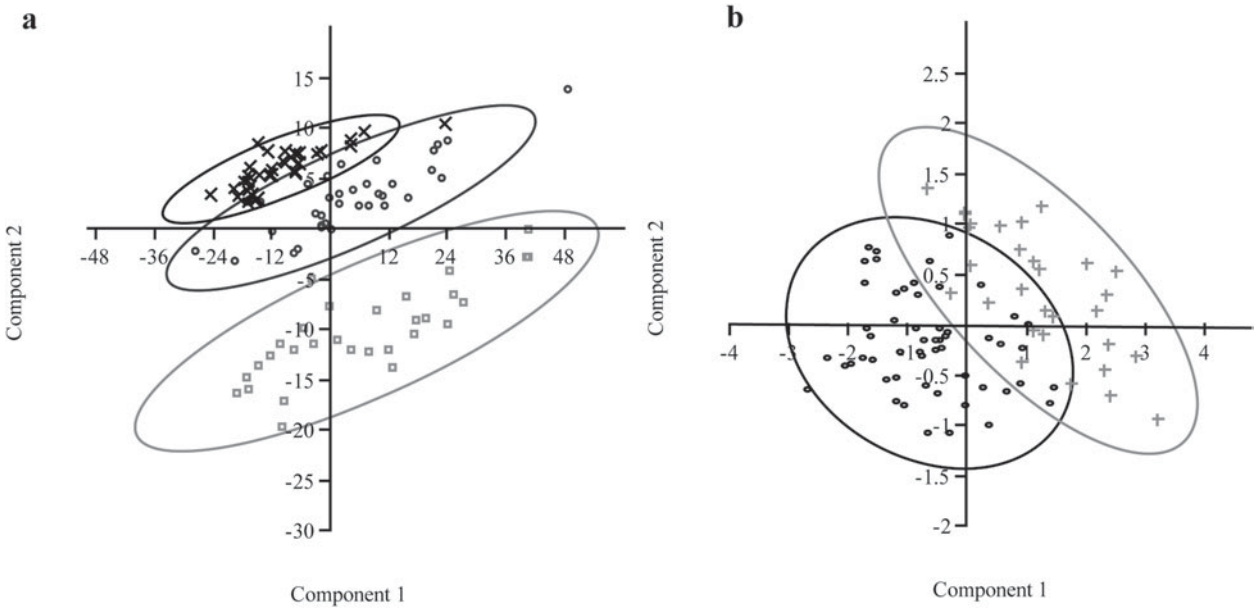

Fig. 4. Principal component analysis (PCA) of morphometric data for (A) *Auerbachia scomberoidi* n. sp. (grey circles), *A. chaetodoni* n. sp. (dark grey crosses) and *A. caranxi* n. sp. (light grey squares); (B) *Coccomyxa colurodontidis* n. sp. (light grey crosses) and *C. gobiodoni* n. sp. (dark grey dots).

GENERAL REMARKS

The 5 new species characterized in this paper are morphologically, genetically, and biologically distinct from all other *Auerbachia* and *Coccomyxa* species described to date. Principal component analysis supports the morphological separation of the species described here (Fig. 4). Discriminant analysis conducted on the morphometric data of the novel *Coccomyxa* spp. indicated that polar capsule length was the character that most contributed to the statistically significant variation observed between these taxa. Total spore length was the character that most contributed to the variation observed between the novel *Auerbachia* spp. To further support the separation of *Coccomyxa* spp. described in this study from those already characterized (shown in Table 2) principal component analysis was also conducted using the minima and maxima as well as average (mean) measurements. This limited analysis (not shown in figures) aided in establishing boundaries between species from confamilial hosts (i.e. *C. gobiodoni* n. sp. and *C. jirilomi* both from hosts within the family Gobiidae).

Molecular analysis included 39 myxosporean sequences, including 1 *Auerbachia* sequence and 2 *Coccomyxa* sequences available from GenBank together with the sequences generated in this study (shown in bold in Fig. 5). Neighbour-joining, Maximum Parsimony and Bayesian analysis (Fig. 5) all produced trees of similar topology characterized by the presence of 6 clades. These clades can broadly be described as a *Ceratomyxa* clade, *Enteromyxum* clade, *Kudoa* clade, *Parvicapsula* clade, *Auerbachia* and *Coccomyxa* clade and a mixed clade containing species of *Myxidium, Zschokkella, Sinuolinea* and *Ellipsomyxa*. All species described in this paper showed high levels of statistical support for close relatedness to other species within the genera *Auerbachia* and *Coccomyxa*.

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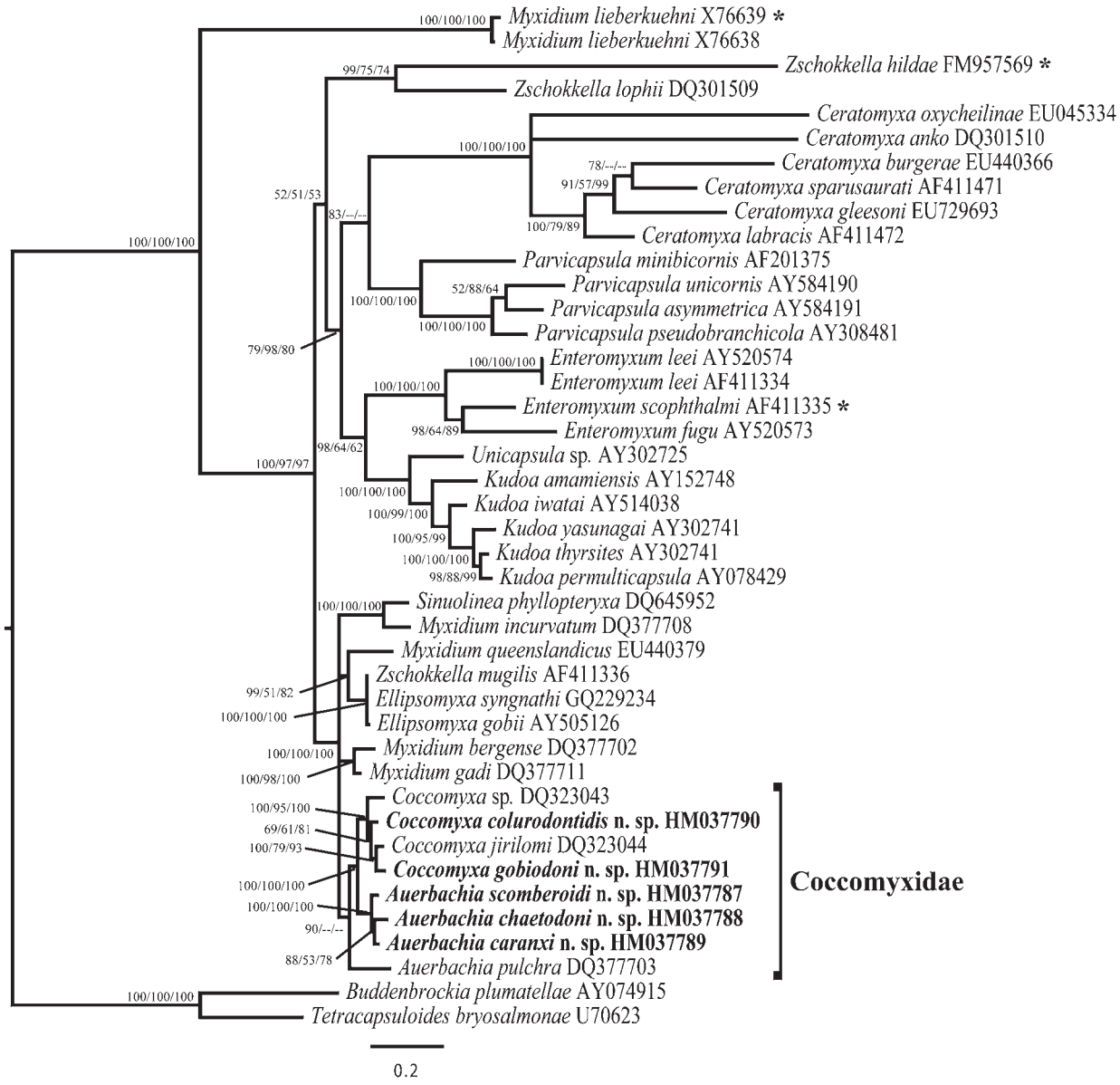

Fig. 5. Phylogenetic tree resulting from Bayesian analysis inferred from the SSUrDNA dataset. Support values at branching points are listed as: Posterior Probabilities (PP) from Bayesian analysis/Bootstrap values from parsimony analysis/Bootstrap values from Neighbour-joining analysis. Any values below 50% are indicated by dashes. GenBank Accession number follows each taxon. Species from this study are shown in bold. Type species for genera currently within the Myxidiidae are indicated with *.

Re-establishment of the family Coccomyxidae Léger & Hesse, 1907

The genus *Auerbachia* was originally established by Meglitsch (1968) who designated *A. anomala* as type species and described *A. montrosa* as a second novel species in the same publication. He did not assign it to any higher taxon at that time. After a short time dwelling in the, now defunct, Myxosomatidae (see Shul'man, 1966), Evdokimova (1973) proposed the new family Auerbachiidae to house her new species, *Auerbachia sphaerica*, and those of Meglitsch (1968). Two years later, Lom *et al.* (1975) re-described *Auerbachia sphaerica* as *Globospora sphaerica,* establishing a new genus. Since then the Auerbachiidae has been recognized in major taxonomic publications (Lom and Noble, 1984; Lom and Dykova, 2006) as a valid family in the Suborder Variisporina containing 2 genera, *Auerbachia* with type species *A. anomala* Meglitsch, 1968 and *Globospora* with type, and only, species *G. sphaerica* (Evdokimova, 1973).

At its proposal (Lèger and Hesse, 1907), the genus *Coccomyxa* was placed in the family Coccomyxidae Léger & Hesse, 1907 which was erected on the basis that their type species, *Coccomyxa morovi*, could not be accepted into the diagnosis of the Myxobolidae, which, at that time, was one of the few myxosporean families in existence. Later, Lom and Noble (1984) placed *Coccomyxa* into the Myxidiidae and amended

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Table 4. Spore measurements for host records of *Auerbachia monstrosa*

(Measurements in μm.)

Species	Host	Geographical location	Total spore length	Spore width	PC length	PC width	CEL
Auerbachia monstrosa Meglitsch, 1968	*Coelorhynchus australis*	New Zealand	25·2 (21·1–28·9)	9·5 (9·3–10·3)	11·2 (9·8–13·2)	4·3 (3·9–4·9)	12·2 (9·8–15·7)
Auerbachia monstrosa of Moser and Noble, 1977	*Coelorhynchus australis* *C. innotabilis*	New Zealand	26·5 (21–32)	9·9 (9–12)	11·5 (9·8–16)	4·6 (3·9–6)	

the family diagnosis to accept a spore with a single polar capsule.

It is clear from our inferred phylogenetic relationship (Fig. 5) that, based on SSU rDNA sequence data, *Coccomyxa* spp. are more closely related to *Auerbachia* spp. than they are to other members of the Myxidiidae (i.e. *Myxidium, Zschokkella* and *Enteromyxum* spp.). These data are unambiguous in demonstrating the paraphyletic nature of the Myxidiidae. Furthermore, we feel it would be incongruous for us to propose new species that show convincing evidence, from both morphological and molecular studies, of their relatedness but then refer them to different families. Currently, the family Auerbachiidae Evdokimova, 1973 is considered valid (see Lom and Dykova, 2006) and includes species in the genera *Auerbachia* and *Globospora*.

Based on priority and on the new data provided in this study, we re-establish Coccomyxidae (Lèger & Hesse, 1907) to house species of the *Coccomyxa* and additionally, we transfer all species of *Auerbachia* and *Globospora* into the Coccomyxidae. We provide a new familial diagnosis for the Coccomyxidae and a list of its genera and species below:

Class Myxosporea Bütschli, 1881
Order Bivalvulida Shulman, 1959
Suborder Variisporina Lom & Noble, 1984
Family Coccomyxidae (Léger & Hesse, 1907)

Diagnosis: Spores with symmetrical or asymmetrical equal or unequal smooth shell valves and a single elongated polar capsule with polar filament making a few longitudinal turns. Coelozoic or histozoic, mono- or polysporic plasmodia of marine fishes.

Coccomyxa Léger & Hesse, 1907
- *C. morovi* Léger & Hesse, 1907 type species.
- *C. baleswarensis* Sarkar, 1995
- *C. claviforme* Cunha & Fonseca, 1919
- *C. jirilomi* Diamant, Lipshitz & Ucko, 2007
- *C. leognathi* Wu, 1991
- *C. meridiei* Lom, Rohde & Dykova, 1992
- *C. tenuiparies* Lom, Rohde & Dykova, 1992
- *C. hoffmani* Cheung & Nigrelli, 1990
- *C. ovale* Kovaljova & Gajevskaja, 1968
- *C. colurodontidis* n. sp. (this study)
- *C. gobiodoni* n. sp. (this study)

Auerbachia Meglitsch, 1968
- *A. anomala* Meglitsch, 1968 type species
- *A. bajadi* Abdel-Baki, 2010
- *A. chakravartyi* Narasimhamurti, Kalavarti, Anuradha & Padma, 1990
- *A. chorinemusi* Padma-Dorothy, Kalavati & Vaidchi, 1998
- *A. hepatica* Sarkar, 2006
- *A. monstrosa* Meglitsch, 1968

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Table 5. Spore measurements for all host records of *Auerbachia pulchra*

(Measurements in μm.)

Species	Host	Geographical location	Spore length	Spore width	PC length	PC width
Auerbachia pulchra Lom, Noble & Laird, 1975	*Macrourus berglax*	Newfoundland, Iceland	30 (26–34)	11 (11–12)	12 (9–14)	4 (3·5–5)
Auerbachia pulchra of Moser and Noble, 1977	*Coelorinchus carminatus*	Florida	24 (18–30)	7·6 (5–10)	11·0 (7·5–15)	4·8 (3–7·5)
	Coelorinchus occa	Lesser Antilles				
	Coryphaenoides filifer	Oregon, Washington, B.C., Canada				
	Coryphaenoides acrolepis	Northern Canada				
	Coryphaenoides pectoralis (=Albatrossia pectoralis)	Northern Canada				
	Macrourus berglax	Iceland, Newfoundland				
	Malacocephalus occidentalis	Columbia				
	Nezumia bairdii	Nova Scotia				

A. pulchra Lom, Noble & Laird, 1975
A. scomberoidi n. sp. (this study)
A. chaetodoni n. sp. (this study)
A. caranxi n. sp (this study)

Globospora Lom, Noble & Laird, 1975
G. sphaerica (Evdokimova, 1973)

DISCUSSION

The 5 new species described in this paper are distinct from all previously described species of *Auerbachia* and *Coccomyxa* and provide the first record of *Auerbachia* and the second record of *Coccomyxa* found in Australian waters. Nonetheless, the diversity and prevalence of both *Auerbachia* and *Coccomyxa* in Australian marine hosts appears to be relatively low.

Before the incorporation of molecular systematics in myxozoan taxonomy, host specificity was difficult to determine. Recent studies on myxozoan host specificity have shown different patterns for different genera. The bivalvulidan genera *Ceratomyxa* and *Myxobolus* tend to be highly host-specific and generally restricted to a single host species, while *Myxidium* and *Kudoa* species may have a broad host range even infecting fishes from different orders (Gunter and Adlard, 2008; Burger and Adlard, 2009; Gunter *et al*. 2009; Molnár *et al*. 2009). As a result no broad trends for host specificity can be adopted within the Myxozoa, but rather, it is important to assess host specificity for each genus independently.

The two new *Coccomyxa* species reported here have each been found only in single host species, similar to all other described species of *Coccomyxa*. However, there are 2 families of teleosts with multiple species of *Coccomyxa* reported. *Coccomyxa baleswarensis, C. meridiei* and *C. morovi* have all been reported to infect 3 different genera of Clupeidae. Nonetheless, all 3 of these species are distinct through morphological comparison of mature spores and plasmodia, as well as geographical location (reported from India, Australia and the Mediterranean Sea, respectively). *Coccomyxa jirilomi* and *C. gobiodoni* n. sp. have been recorded from 2 genera of Gobiidae. Although these species are superficially similar in morphology they are both genetically (22 bp differences, 98·2% similarity) and geographically (reported from Israel and Australia, respectively) distinct.

The 3 new species of *Auerbachia* reported here have, like most previously described species, each been found only in single host species. Two exceptions include *A. pulchra* (Moser and Noble, 1977; Zubchenko, 1985; Khan *et al*. 1986; Fiala, 2006) and *A. monstrosa* (see Moser and Noble, 1977; Walter *et al*. 2002) that have been reported from several host species from several host families (Table 4). Equally, the occurrence of these two species within more than a single host species may be the result of insufficient morphological resolution to discriminate between the type species and closely related species. As such, they could represent the presence of a species complex rather than truly reflecting a broad host range.

Auerbachia monstrosa was originally described by Meglitsch (1968) from *Coelorinchus australis* (Richardson, 1839) (reported as *Coelorhynchus australis*) from New Zealand waters. Moser and Noble (1977) detected the species in the type host

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and a second *Coelorinchus* species, *C. innotabilis* McCulloch, 1907 from the same region. The morphometrics from both hosts were combined ($n = 26$ spores) and the means and ranges were superficially similar to Meglitsch's original description (Table 4). The ranges reported by Moser and Noble (1977) are larger, perhaps due to combining measurements from both hosts. Unfortunately, any data on slight morphometric differences occurring between isolates from these closely related hosts are not available. Walter *et al*. (2002) also reported *A. monstrosa* in another macrourid fish, *Macrourus whitsoni*. No morphometrics were given and it appears that the diagnosis was based on host range. Walter *et al*. (2002) stated "According to Moser and Noble (1977), *Auerbachia* spp. occur in several widely distributed fish species, such as *Coelorhinchus caelorhincus* (Risso, 1810) and *Macrourus berglax* Lacapède, 1810. The present finding of *Auerbachia monstrosa* in *Macrourus whitsoni* represents a new host record". However, the hosts listed above represent hosts for *A. pulchra* not *A. monstrosa*. This example highlights the contentious nature of species diagnostics and its influence on our understanding of host specificity.

The reported host range of *A. pulchra* is just as contentious. This species was first described by Lom *et al*. (1975) in *Macrourus berglax* off Newfoundland and Iceland. Morphometric differences could differentiate this species from all other *Auerbachia* including *A. monstrosa*, which also infects fishes from the Macrouridae. The species boundary becomes harder to distinguish when the host range was extended to include a further 8 hosts all of which are macrourids (Moser and Noble, 1977). Again the morphometrics of spores from these 8 hosts were combined for a total of 190 spores. However, the measurements given by Moser and Noble (1977) appear to be significantly different from the original report (Table 5). To add to the confusion, the morphometrics of *A. monstrosa* described by Moser and Noble (1977) overlap with these measurements. It appears that species diagnosis by Noble and Lom (1975) is based on locality and host range, with *A. monstrosa* being found only off New Zealand and restricted to fishes from the macrourid genus *Coelorhinchus*. Regardless, the species boundary between *A. monstrosa* and *A. pulchra* needs further investigation. Khan *et al*. (1986) carried out a comprehensive morphometric analysis using both fresh and preserved material of *Auerbachia* from *Macrourus berglax* caught in the eastern Weddel Sea. This isolate was diagnosed as *A. pulchra* because spore measurements were consistent with those previously recorded and the material was collected from the type host. Fiala (2006) reported *A. pulchra* from a new host *Coryphaenoides rupestris* from the North Atlantic, no morphometrics are given for this species and it was possibly assigned to that species due to the reported broad host range which includes other fishes from the same genus. The isolate from *Coryphaenoides rupestris* has corresponding SSU rDNA sequence data and will be useful for investigating whether *A. pulchra* represents a species complex.

Two of the 3 *Auerbachia* species described here are from fishes from different genera of the family Carangidae both collected off Lizard Island, GBR. These 2 isolates are genetically distinct (15 bp differences, 98·8% similarity). While the level of genetic variation between species is not consistent across different taxonomic groups, intra-specific variation in other myxozoan genera has been reported at similar, relatively low levels (e.g. 1·3% for *Ceratomyxa* and 1% for *Kudoa*). On the basis of morphometric differences supported by principle component analysis together with the observed genetic differences, we are confident that these represent different species. Additionally, 3 other species of *Auerbachia* have been reported from carangids. *Auerbachia hepatica* was described from *Carangoides praeustus* in India (Sarkar, 2006) and can be distinguished from both species on the basis of site of infection and on spore morphology. *Auerbachia chorinemusi* was described from *Scomberoides tol* (= *Chorinemus tol*) in India (Padma-Dorothy *et al*. 1998) but it can easily be distinguished from *A. scomberoidi* n. sp. on the basis of morphometric differences, as can *A. bajadi* from *Carangoides bajad*. *Auerbachia chorinemusi* and *A. caranxi* n. sp. are superficially similar except that the polar capsule of *A. chorinemusi* is wider and the caudal extension is shorter than that reported here for *A. caranxi* n. sp. Once SSU rDNA sequence data corresponding to *A. chorinemusi* are available we can be more certain of its identity. However, we feel it is more useful taxonomically to separate the species of *Auerbachia* that infect the gall bladders of carangid fishes on the basis of morphometric, geographical and host differences than to potentially create a species complex within *A. chorinemusi*.

Molecular information is becoming an invaluable tool for species discrimination, especially for bivalvulidan species of parasites which, like many other taxa, often have limited morphological characters that are taxonomically informative at the light microscope level and furthermore, many characters display plasticity (Sitja-Bobadilla and Alvarez-Pellitero, 1993; Heiniger *et al*. 2008). Molecular information was used in this study to separate the 3 superficially similar *Auerbachia* isolates and 2 *Coccomyxa* isolates to further support the proposal of these 5 new species.

The molecular analyses included 42 myxozoans (including 4 species of *Auerbachia* and 4 species of *Coccomyxa*) and were primarily aimed at species discrimination then secondarily at phylogenetic relatedness. The *Auerbachia* and *Coccomyxa* species formed a well-supported clade with *Sinuolinea phyllopteryxa, Ellipsomyxa gobii, E. syngnathi, Zschokkella mugilis, Myxidium incurvatum, M. gadi,*

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M. bergense, and *M. queenslandicus*. Of particular significance, the 3 *Auerbachia* species described here formed a well-supported clade with all species of *Coccomyxa* and did so to the exclusion of *A. pulchra*. This relationship is further supported by the pairwise base differences between these species which showed *A. pulchra* was genetically the least similar, with 74–88 bases difference, to the 5 new species described herein, with species of *Auerbachia* being genetically more similar to *Coccomyxa* than to *A. pulchra*.

The genetic distance between *Auerbachia pulchra* and the remaining species of *Auerbachia* and *Coccomyxa* may correlate with host differences. *Auerbachia pulchra* is reported from the deep-sea fishes, *Macrourus berglax* and *Coryphaenoides rupestris,* while all other *Auerbachia* and *Coccomyxa* species analysed here are reported from coral reef-dwelling, shallow-water fish. Similarly, *Ceratomyxa* from deep sea fishes sequenced by Fiala (2006) sit basally to the rest of the clade. Regardless of what drives these relationships, it is clear that our phylogenetic analyses support a close taxonomic relationship between *Auerbachia* and *Coccomyxa,* a relationship which is also reflected in the morphological similarities between these two genera. For example, *Coccomyxa leiognatha,* is morphologically very similar to *Auerbachia* with spores that are club-like in shape complete with a caudal extension (Wu, 1991). Equally clearly, our phylogenetic analyses infer that *Auerbachia* may not be a monophyletic genus and the systematic placement of species in both *Auerbachia* and *Coccomyxa* may be contentious. However, bootstrap support is equivocal and until data are more comprehensive and the type species for both genera have been genetically characterized, we feel that any attempted revision would at this stage be premature.

Nonetheless, the obvious genetic and morphological affinity shown by species of *Coccomyxa* and *Auerbachia* has prompted us to re-establish the Coccomyxidae to house them and, by association, house the monotypic genus *Globospora*. *Coccomyxa* spp. have recently resided in the Myxidiidae which also includes species from the genera *Myxidium, Enteromyxum* and *Zschokkella*. Included in our analyses are the type species of these genera (*M. lieberkueni, E. scophthalmi* and *Z. hildae*) and their relative, and well-supported, positions in the phylogeny demonstrate unequivocally that the Myxidiidae is not a natural grouping. The Auerbachiidae was first proposed by Evdokimova 1973 but the Coccomyxidae Léger and Hesse, 1907 clearly has priority as a familial concept.

We undertake this nomenclatural change as an attempt to stabilize the taxonomy of a small group of genera and species within the Bivalvulida and recognize that a considerable effort remains to clarify relationships within this myxozoan taxon.

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