Low host specificity in the Kudoidae (Myxosporea: Multivalvulida) including seventeen new host records for *Kudoa thalassomi*

Mieke A.A. Burger^{1,2} and Robert D. Adlard^{1,2}

¹ School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, Queensland 4072, Australia;

² Biodiversity Program, Queensland Museum, South Brisbane, Queensland 4101, Australia

Abstract: Kudoid parasites are known to infect a large variety of fish. A significant proportion of *Kudoa* species have relatively low host specificity, with a single species able to infect multiple host species representing various host families even from different host orders. Since DNA sequences have been associated with myxosporean species characterisations, it has become far easier to determine host range of new species and validate host records from earlier descriptions. This study investigated the host specificity of a kudoid parasite, *Kudoa thalassomi* Adlard, Bryant, Whipps et Kent, 2005, from the Great Barrier Reef in Australia using DNA sequence analysis and morphology. The results revealed the host specificity to be broad, with *K. thalassomi* identified in 18 different fish species representing six different fish families. This study also compares current genetic information from different host isolates of *Kudoa* Meglitsch, 1947 to their host ranges recorded in existing literature. From this analysis, only half of the *Kudoa* species with multiple host records (27 *Kudoa* species) have half or more isolates that are genetically characterised, and thus specifically identified with a high confidence, from their known hosts. Only five kudoid species have genetically characterised isolates from all of their recorded hosts.

Keywords: Myxosporea, Kudoidae, host specificity, *Kudoa thalassomi*, genetic variation

Parasites from the monotypic family Kudoidae Meglitsch, 1960 are microscopic, spore-forming organisms found commonly in marine fish. Some of these parasites are of significant concern to marine aquaculture through their production of post-mortem myoliquefaction, encephalomyelitis, and obvious cysts in the musculature (Egusa 1986a, Alvarez-Pellitero and Sitja-Bobadilla 1993, Moran et al. 1999, Grossel et al. 2003). However, some kudoid species seem to cause relatively little effect in some hosts while causing significant issues in other hosts, e.g. *Kudoa amamiensis* Egusa et Nakajima, 1980 in pomacentrid fish (Egusa and Nakajima 1980, Burger et al. 2008) compared to *Seriola* spp.

Clearly, the presence of kudoid parasites can impact on aquaculture production, with the host specificity of such parasites being a key element required to determine the actual level of threat. Host species-specific kudoids that occur in non-commercial fish obviously pose little threat, while kudoids with broader specificity may transfer to cultured stock through the presence of locally occurring reservoir hosts. Most myxosporeans have been reported from a single host species or host genus (Moran et al. 1999) but many kudoid species tend to show lower host specificity. For example, *Kudoa iwatai* Egusa et Shiomitsu, 1983 has been recorded from 13 different

fish families from three fish orders (Egusa and Shiomitsu 1983, Sugiyama et al. 1999, Diamant et al. 2005), and the cosmopolitan *K. thyrssites* (Gilchrist, 1924) has been recorded from 18 different fish families representing nine fish orders (Whipps and Kent 2006). Recent trends to incorporate genetic data in species descriptions facilitates the unambiguous identification of infections of described kudoid species occurring in new hosts, regardless of phenotypic plasticity, and equally, it facilitates the recognition of novelty in typically character-poor, morphologically similar, species. This study compares the host range of kudoid species determined by molecular diagnosis with those that were determined prior to, or without the aid of DNA sequences, and reports 17 new host records for *Kudoa thalassomi* Adlard, Bryant, Whipps et Kent, 2005.

MATERIALS AND METHODS

Sample collection. Fish from 214 species of 41 fish families were collected (during 2005–2008) from Heron Island (23°27'S, 151°55'E) and Lizard Island (14°39'S, 145°7'E) on the Great Barrier Reef, Queensland, Australia. Fish were caught using line, spear, or localised sprays of clove oil anaesthetic and euthanised by prolonged immersion in clove oil. Identification of fish hosts conforms to currently valid names and common names given in FishBase (<http://www.fishbase.org/>).

The fish were examined for kudoid infection in the somatic muscle by taking a sub-sample of fish tissue (approximately 20 × 10 × 5 mm) that was placed on a glass plate (76 × 50 mm), moistened with vertebrate saline (0.85% salt), and shredded to a pulp using scalpel blades. A second glass plate was placed on top and the liquid squeezed out into a 2-ml centrifuge tube. An aliquot (~15 µl) of the suspension from the bottom of the tube was examined for kudoid spores under an Olympus BH2 microscope at ×400 magnification. When kudoid spores were detected, sub-samples of skeletal muscle were preserved in 10% neutral buffered formalin for histological analysis and the remainder of the fish and the saline preparation were preserved by freezing for morphological analysis and DNA extraction of spores. Where material was limited, priority went to frozen samples from which both morphological information and DNA could be recovered.

Histology. Sections (5 µm thick) were cut from selected 10% formalin-fixed samples using standard histological techniques. Serial sections were stained alternatively with haematoxylin and eosin or with Giemsa and eosin, and coverslips applied using DePeX (BDH, England). Digital, light photomicrographs of the sections were taken at ×400 magnification with an Olympus BH2 microscope using a Nikon Digital Sight DS-L1 (Nikon Corporation, Japan) camera.

Morphometric analysis. Spore suspensions in saline were prepared as described above from two selected fish from two different families: *Abudefduf bengalensis* (Bloch) (Pomacentridae) from Heron Island and *Thalassoma lunare* (Linnaeus) (Labridae) from Lizard Island. Photomicrographs were taken of the spores at ×400 magnification under phase contrast lighting conditions. Spore measurements were taken from the micrographs using the measuring tool in Nikon NIS Elements imaging software (Nikon, Tokyo). At least 30 spores from each morphotype in apical view were used to represent the measurements taken (side view measurements were taken from the *A. bengalensis* sample only).

The morphological data were subjected to a principal component analysis using the software PAST version 1.82b (Hammer et al. 2001). Scatter plots with 95% confidence ellipses were generated using variant-covariant matrices. All other statistical analysis was conducted using Stata™ 10.0 (StataCorp 2008). The raw data of each measurement were tested for normal distribution using the Shapiro-Wilk test with a 95% confidence level. Each normally distributed dataset was tested individually for significant difference between the means from two host isolates first by Bartlett's test for equal variance (one-way analysis of variance), followed by two-sample *t*-test (for either equal or unequal variance depending on the outcome of the Bartlett's test).

DNA analysis. DNA was extracted from *Kudoa* spore suspensions that had been examined under the microscope during sample collection. Kudoids were sampled from 18 fish species in six different host families: *Cheilodipterus macrodon* (Lacépède) (Apogonidae); *Chaetodon baronessa* Cuvier, three *C. unimaculatus* Bloch individuals, *C. vagabundus* Linnaeus, *Chelmon rostratus* (Linnaeus), and *Heniochus monoceros* Cuvier (Chaetodontidae); *Thalassoma lunare* and *T. lutescens* (Lay et Bennett) (Labridae); *Abudefduf bengalensis*, *A. whiteleyi* Allen et Robertson, two *Amblyglyphidodon curacao* (Bloch) individuals, *Amphiprion akindynos* Allen, *A. melanopus*

Bleeker, *Chrysiptera cyanea* (Quoy et Gaimard), *Dascyllus aruanus* (Linnaeus), and *Neoglyphidodon melas* (Cuvier) (Pomacentridae); *Scarus flavipectoralis* Schultz (Scaridae); and *Cephalopholis boenak* (Bloch) (Serranidae). The spore suspensions were concentrated by centrifugation for 6 min at 4750 *g* and the supernatant discarded. The DNA from the pellet was extracted using a QIAgen DNeasy Kit (QIAGEN Inc., Valencia, California) according to the manufacturer's instructions. Small subunit (SSU, 18S) rDNA was amplified using PCR primers KudMB1f 5'-GCC ATG GAT AAC TGT GGT AAA TCT AGA GC -3' (Burger et al. 2008) and universal 18R 5'-CTA CGG AAA CCT TGT TAC G -3' (Whipps et al. 2003). Large subunit (LSU, 28S) rDNA was amplified using PCR primer combination Kt28S1f 5'-CAA GAC TAC CTG CTG AAC -3' and 28S1R 5'-GTG TTT CAA GAC GGG TCG -3' (Whipps et al. 2004). PCR reactions and cycling parameters were as per Burger et al. (2008). PCR products were cloned using TOPO TA Cloning® Kit for Sequencing (Invitrogen, Carlsbad, CA, USA) as per the manufacturer's instructions and DNA sequence data were generated as described in Burger et al. (2008). One clone of LSU was sequenced for each host, while two clones were sequenced from the LSU PCR product from *Amphiprion akindynos* and two clones from the second *Chaetodon unimaculatus* individual.

SSU rDNA and LSU rDNA sequence data were edited using BioEdit (Hall 1999) then aligned with other kudoid sequences available from GenBank using ClustalW (Thompson et al. 1994) and subsequent neighbour joining distance analyses using the p-distance model were conducted using PAUP* 4.0b10 (Swofford 2002). The SSU *Kudoa rosenbuschi* (Gelormini, 1943) sequence from Abollo et al. (2005) and the *Kudoa* sp. sequences from Pascual and Abollo (2008) were considered as sequences of *Kudoa alliaria* Shulman et Kovaleva, 1979 as discussed in Burger and Adlard (2010). *Unicapsula* sp., a multivalvulidan in the family Trilosporidae, was used as an outgroup because it represents the closest known relative external to the Kudoidae. The bootstrap confidence values were generated using 1000 bootstrap replicates. Pairwise differences were also analysed in PAUP* 4.0b10 to determine total nucleotide distance and percentage differences.

Host specificity. A survey of the literature was conducted to compare host ranges amongst the Kudoidae Meglitsch, 1960 (amended by Whipps et al. 2004). All records were compared to availability of genetic data correlating to *Kudoa* species with multiple hosts. While every effort has been made to include all *Kudoa* species with multiple hosts and to include all their host records, it is possible that some in peripheral literature may have been overlooked. The authors welcome any further contributions.

RESULTS

Identity of new *Kudoa* isolates

New isolates of *Kudoa* were collected from the somatic muscle from 18 species of six different fish families on the Great Barrier Reef, Queensland, Australia. The *Kudoa* samples from these fish had spores with 6 or 7 polar capsules and spore valves within a single plasmodium (Figs. 1, 2). As such, from microscopic observation all

isolates were morphologically consistent with the original description of *Kudoa thalassomi* Adlard, Bryant, Whipps et Kent, 2005.

The genetic sequence data produced from the 18 isolates in this study possess the highest genetic affinity with *K. thalassomi* ex *Thalassoma lunare* (minimum 99.7% affinity in SSU (range of 0–10 nucleotides difference) and 98.5% in LSU (range of 1–10 nucleotides difference)) (see Figs. 3, 4). The neighbour joining trees (Figs. 3, 4) show no correlation between host relatedness and sequence affinity between host isolates. Interestingly, 4 nucleotides difference (0.6%) was found in LSU between the type sequence of *Kudoa thalassomi* and the *Kudoa* sequence from the same host, *T. lunare* from Lizard Island.

To examine the breadth of morphological variation, two of these *Kudoa* isolates were subjected to more detailed morphological analysis. The first was an isolate from the type host of *Kudoa thalassomi*, *Thalassoma lunare* (Labridae), while the second was an isolate recovered from *Abudefduf bengalensis* representing a different and more taxonomically distant host family (Pomacentridae) of coral reef associated fishes. All measurements conformed to a normal distribution using the Shapiro-Wilk test (p -value >0.05). A principal components analysis comparing the apical view measurements of the 6- and 7-polar capsule morphotypes from these two host isolates (Fig. 5) shows overlap between all the morphotypes, but reveals significant differences between the two isolates and between the two different morphotypes. This is especially obvious between the two 7-polar capsule morphotypes (Fig. 5). The two sample t -test identified significant differences (p -value <0.05) in the means between the 7-polar capsule morphotype from the different hosts in width, valve width, and apical polar capsule length. For the 6-polar capsule morphotype, both the spore width and the valve width have significantly different means.

Compared to other species that infect somatic muscle and are dominated by 6-polar capsule spores (see Table 1), the two new *Kudoa* isolates are closest in size, but generally slightly larger than those recorded from *K. thalassomi* described by Adlard et al. (2005). The exception being for the side view polar capsule length, where the *K. thalassomi* from Adlard et al. (2005) is larger (mean 4.9 μm cf. 4.3 μm) than that from *Abudefduf bengalensis* from this study. There is minimal overlap in the ranges for the thickness, length, and side view polar capsule width between the new isolates and *K. thalassomi*. The new *Kudoa* isolates are easily distinguished from *K. grammatorcyni* Adlard, Bryant, Whipps et Kent, 2005 and *K. scomberomori* Adlard, Bryant, Whipps et Kent, 2005 and they are generally larger than *K. neothunni* (Arai et Matsumoto, 1953), but there is a small overlap in size ranges in width, length, and side view polar capsule width. *Kudoa neothunni* has only been recorded from yellowfin tuna, *Thunnus albacares* (Bonnaterre) from Japan and is

Table 1. Morphometric comparison (mean, standard deviation and range in parentheses) between the 6- and 7-polar capsule morphotypes of the two new *Kudoa thalassomi* isolates with the original measurements from Adlard et al. (2005) and with three other species containing six polar capsules, known to infect skeletal muscle. PC = polar capsule.

<i>Kudoa</i> species Study	Host	No. PCs	Apical view measurements (μm)				Side view measurements (μm)				
			Width	Thickness	Valve width	Suture radius	Apical PC length	Apical PC width	Length	Side PC length	Side PC width
<i>K. thalassomi</i> This study	<i>Abudefduf bengalensis</i>	6	13.67, 0.59 (12.4–14.9)	11.52, 0.51 (10.4–12.6)	6.84, 0.30 (6.2–7.5)	4.57, 0.21 (4.3–5.1)	3.60, 0.26 (3.1–4.1)	2.1, 0.13 (1.7–2.4)	7.26, 0.34 (6.7–7.8)	4.28, 0.18 (3.9–4.6)	2.01, 0.19 (1.6–2.3)
		7	14.27, 0.47 (12.9–15.0)	–	7.37, 0.33 (6.7–7.9)	4.80, 0.24 (4.1–5.2)	3.88, 0.23 (3.3–4.3)	2.12, 0.11 (1.9–2.3)	–	–	–
	<i>Thalassoma lunare</i>	6	13.16, 0.59 (12.3–14.9)	11.70, 0.6 (10.5–13.5)	6.56, 0.35 (5.5–7.5)	4.81, 0.17 (4.5–5.2)	3.56, 0.18 (3.2–4.0)	2.12, 0.07 (2.0–2.2)	–	–	–
		7	13.48, 0.69 (12.3–14.9)	–	6.76, 0.39 (6.1–7.7)	5.13, 0.26 (4.6–5.8)	3.63, 0.28 (3.2–4.2)	2.13, 0.06 (2.0–2.3)	–	–	–
<i>K. thalassomi</i> Adlard et al. 2005	<i>Thalassoma lunare</i>	6	10.658 (9.474–11.842)	9.37 (8.553–10.789)	–	3.987 (3.421–4.408)	–	–	6.645 (6.184–7.105)	4.921 (4.737–5.000)	2.123 (2.039–2.237)
<i>K. grammatorcyni</i> Adlard et al. 2005	<i>Grammatocynus bicarinatus</i>	6	8.623 (8.026–8.947)	8.14 (7.632–8.684)	–	3.881 (3.619–4.094)	–	–	6.535 (6.316–6.711)	3.684 (3.553–3.861)	1.721 (1.645–1.842)
	<i>Neothunnus macropterus</i>	6	11 (9.1–13.0)	–	–	3.6 (3.0–4.4)	–	–	6.2 (5.3–7.3)	2.5 (2.0–3.1)	1.6 (1.3–2.1)
<i>K. neothunni</i> Arai and Matsumoto 1953											
<i>K. scomberomori</i> Adlard et al. 2005	<i>Scomberomorus commerson</i>	6	7.555 (6.842–8.158)	6.794 (6.184–7.632)	–	2.959 (2.632–3.158)	–	–	5.425 (5.0–6.184)	3.235 (3.026–3.553)	1.371 (1.25–1.513)

a species that causes liquefaction of the somatic muscle (Arai and Matsumoto 1953).

From here on, the new *Kudoa* isolates will be referred to as *K. thalassomi* due to their high genetic affinity with this species, similar geographic locality (on the Great Barrier Reef) to where the original type specimen was collected, and their clear distinction in both genetics and morphology to other superficially similar kudoids described to date.

Kudoa thalassomi Adlard, Bryant, Whipps et Kent, 2005

Type host: *Thalassoma lunare* (Linnaeus), moon wrasse (Teleostei, Labridae).

Type locality: Swain Reefs (21°36.20'S, 152°22.26'E), Great Barrier Reef, Queensland, Australia.

Site of infection: Somatic muscle, pseudocysts not evident macroscopically.

New material for *Kudoa thalassomi*: See Figs. 1 and 2.

Notes: Plasmodia microscopic, polysporic. No membrane or inflammatory response evident. Spores of uniform development, extra-sporogonic stage not observed. Dominant spore morphotype has six polar capsules and spore valves, minor spore morphotype has seven (Fig. 2). Radially symmetrical in apical view. Polar capsules convergent, pyriform. In side view, spores are pyramidal. Spore measurements are shown in Table 1.

Other hosts: *Cheilodipterus macrodon* (Lacépède) – large toothed cardinalfish (Apogonidae); *Chaetodon baronessa* Cuvier – eastern triangular butterflyfish, *Chaetodon unimaculatus* Bloch – teardrop butterflyfish, *Chaetodon vagabundus* Linnaeus – vagabond butterflyfish, *Chelmon rostratus* (Linnaeus) – copperband butterflyfish, *Heniochus monoceros* Cuvier – masked bannerfish (Chaetodontidae); *Thalassoma lutescens* (Lay et Bennett) – yellow-brown wrasse (Labridae); *Abudefduf bengalensis* (Bloch) – Bengal sergeant, *Abudefduf whitleyi* Allen et Robertson – Whitley's sergeant, *Amblyglyphidodon curacao* (Bloch) – staghorn damselfish, *Amphiprion akindynos* Allen – barrier reef anemone fish, *Amphiprion melanopus* Bleeker – fire clownfish, *Chrysiptera cyanea* (Quoy et Gaimard) – sapphire devil, *Dascyllus aruanus* (Linnaeus) – whitetail dascyllus, *Neoglyphidodon melas* (Cuvier) – bowtie damselfish (Pomacentridae); *Scarus flavipectoralis* Schultz – yellowfin parrotfish (Scaridae); *Cephalopholis boenak* (Bloch) – chocolate hind (Serranidae).

Other localities: Heron Island (23°27'S, 151°55'E) and Lizard Island (14°39'S, 145°27'E) on the Great Barrier Reef, Queensland, Australia.

New material: Giemsa and eosin-stained histological section vouchers deposited in the collections of the Queensland Museum, Brisbane, Australia: ex *Neoglyphidodon melas*, G465422; ex *Amphiprion akindynos*, G465423; ex *Dascyllus aruanus*, G465424; ex *Abudefduf sexfasciatus*, G465425. Haematoxylin and eosin-stained histological section vouchers: ex *Neoglyphidodon melas*, G465426; ex *Amphiprion akindynos*, G465427; ex *Dascyllus aruanus*, G465428; ex *Abudefduf sexfasciatus*, G465429. GenBank sequence

Fig. 1. Giemsa and eosin-stained histological section through a skeletal muscle fibre infected with *Kudoa thalassomi*. Both 6- and 7-polar capsule morphotypes are visible.

numbers HM022110–HM022116 for SSU and HM022117–HM022139 for LSU (see Figs. 4 and 5 for specific isolate numbers).

***Kudoa* species with multiple hosts**

Of the 84 *Kudoa* species described to date, 27 of them have been associated with more than one host with a median of four hosts for *Kudoa* species with more than one host. Eight of these 27 *Kudoa* species only have two known hosts (*K. azoni* Aseeva, 2004, *K. camarguensis* Pampoulie, Marques, Rosecchi, Crivelli et Bouchereau, 1999, *K. lethrini* Burger, Cribb et Adlard, 2007, *K. megacapsula* Yokoyama et Itoh, 2005, *K. miniauriculata* Whitaker, Kent et Sakanari, 1996, *K. shiomitsu* Egusa et Shiomitsu, 1983, *K. trifolia* Holzer, Blasco-Costa, Sarabeev, Ovcharenko et Balbuena, 2006, and *K. unicapsula* Yurakhno, Ovcharenko, Holzer, Sarabeev et Balbuena, 2007) while *K. thyrsites* has the highest number of host species recorded at 38, followed by *K. nova* Naidjenova, 1975 with 20, *K. iwatai* with 19, and now *K. thalassomi* with 18. At higher taxonomic levels, 12 of the 27 species are restricted to a single host family and 20 of the 27 are restricted to a single fish order. Fig. 6 shows the frequency distribution of host species range in the Kudoidae. It shows a positively skewed proportion where most species

Fig. 2. Phase contrast photomicrographs of spores from *Kudoa thalassomi* ex *Abudegduf bengalensis*. **A** – 6-polar capsule morphotype; **B** – 7-polar capsule morphotype; **C** – side view of spore.

(57 of 84) have only been recorded from a single host and eight species have been recorded from only two hosts.

Following are data from the literature on the 27 species of kudoids that have been recorded from multiple hosts. Those hosts with corresponding rDNA sequence of their *Kudoa* isolates are highlighted in bold.

Kudoa alliaria Kovaleva, Shulman et Yakovlev, 1979

Total number of hosts: 6. Gadiformes: Gadidae – *Micromesistius australis* (type host). Merlucciidae – ***Macruronus magellanicus***, ***Merluccius australis***, ***Me. hubbsi***. Perciformes: Nototheniidae – *Patagonotothen ramsayi* (*Notothenia ramsayi* in Kovaleva et al. 1979), *Notothenia conina*.

References: Kovaleva et al. 1979, Abollo et al. 2005, Whipps and Diggles 2006, Pascual and Abollo 2008, Burger and Adlard 2010.

Kudoa amamiensis Egusa et Nakajima, 1980

Total number of hosts: 11. Perciformes: Carangidae – ***Caranx sexfasciatus***, *Seriola dumerili*, ***S. quinqueriata*** (type host). Pempheridae – ***Pempheris ypsilychnus***. Pomacentridae – ***Abudegduf bengalensis***, *A. sexfasciatus*, *A. vaigiensis*, ***A. whiteyi***, *Chromis chrysura* (syn. *Chromis isharai* in Egusa and Nakajima 1980), *Chromis notata*, *Chrysiptera cyanea*.

References: Egusa and Nakajima 1980, Hervio et al. 1997, Sugiyama et al. 1999, Whipps et al. 2003, Burger et al. 2008.

Kudoa azoni Aseeva, 2004

Total number of hosts: 2. Scorpaeniformes: Hexagrammidae – *Hexagrammos octogrammus*, *Pleurogrammos azonus* (type host).

Reference: Aseeva 2004.

Kudoa bora (Fujita, 1929)

Total number of hosts: 3. Mugiliformes: Mugilidae – *Mugil carinatus*, *M. cephalus*, *M. japonicus* (type host).

Reference: Fujita 1929.

Kudoa camarguensis Pampoulie, Marques, Rosecchi, Crivelli et Bouchereau, 1999

Total number of hosts: 2. Perciformes: Gobiidae – *Pomatoschistus microps* (type host), *P. minutus*.

Reference: Pampoulie et al. 1999.

Kudoa ciliatae Lom, Rohde et Dyková, 1992

Total number of hosts: 3. Perciformes: Sillaginidae – *Sillago analis*, ***S. ciliata*** (type host), *S. maculata*.

References: Lom et al. 1992, Hallett et al. 1997, Burger et al. 2007.

Kudoa clupeidae (Hahn, 1917)

Total number of hosts: 11. Clupeiformes: Clupeidae – *Alosa aestivalis* (*Pomolobus aestivalis* in Hahn 1917), *A. mediocris*, *A. pseudoharengus* (*Pomolobus pseudoharengus* in Hahn 1917), ***Brevoortia tyrannus***, *Clupea harengus* (type host). Perciformes: Labridae – *Tautoglabrus adspersus*. Pomatomidae – *Pomatomus saltatrix* (syn. *Temnodon saltator* in Balozet 1929, 1930). Scombridae – *Thunnus thynnus*. Sparidae – *Stenotomus chrysops*. Zoarcidae – *Macrozoarces americanus*. Pleuronectiformes: Pleuronectidae – *Platichthys flesus*.

References: Hahn 1917, Balozet 1929, 1930, Nigrelli 1946, Meglitsch 1947, Dollfus 1955, Sindermann 1961, Kovaleva et al. 1979, Pellizzato and Canzonier 1985, Shulman 1988, Reimschuessel et al. 2003, Webb et al. 2005.

Kudoa gunterae Burger et Adlard, 2010

Total number of hosts: 11. Perciformes: Apogonidae – ***Apogon properuptus***. Pomacentridae – ***Abudegduf septemfasciatus***, ***A. sordidus***, ***A. sexfasciatus*** (type host), ***Chromis viridis***, ***Chrysiptera cyanea***, ***Dascyllus aruanus***, ***Dischistodus pseudochrysopoecilus***, ***Neoglyphidodon melas***, ***Plectroglyphidodon leucozonus***, ***Pomacentrus chrysurus***.

Reference: Burger and Adlard 2010.

<i>Kudoo</i> species (no. sequences)	No. species /families	max nt variance	max % variance
<i>Kudoo allaria</i> (12)	3/1	7	0.5
<i>Kudoo lethrini</i> (2)	2/1	0	0
<i>Kudoo thalassomi</i> (8)	8/5	10	0.7
<i>Kudoo gunterae</i> (6)	6/1	8	0.6
<i>Kudoo whipsi</i> (4)	4/2	6	0.4
<i>Kudoo megacapsula</i> (2)	2/2	2	0.1
<i>Kudoo thyrites</i> (18)	14/13	14	0.9
<i>Kudoo paraquadriscornis</i> (5)	4/1	0	0
<i>Kudoo iwatai</i> (8)	8/3	4	0.3
<i>Kudoo lehti</i> (2)	2/1	1	0.1
<i>Kudoo amamiensis</i> (12)	5/3	7	0.5
<i>Kudoo hypoeipicalialis</i> (6)	6/6	0	0
<i>Kudoo shiomi</i> (2)	2/2	0	0

Kudoo species (no. sequences)	No. species /families	max nt. variance	max % variance
<i>Kudoo thalassomi</i> (24)	13/6	14	2.1
<i>Kudoo thyrates</i> (15)	11/10	59	9.2
<i>Kudoo whips</i> (9)	6/2	13	1.8
<i>Kudoo gunterae</i> (12)	10/1	6	0.8
<i>Kudoo paraquadriconis</i> (4)	3/1	0	0
<i>Kudoo amamiensis</i> (5)	3/2	5	0.7
<i>Kudoo kenti</i> (4)	3/1	5	0.7

Fig. 4. Neighbour joining distance analysis of LSU rDNA from *Kudoo* species including table summarising species with sequences from multiple hosts. The number of sequences in the analysis is shown in parentheses; the number of fish families these sequences come from; and the maximum variance in nucleotides and percent are shown. Bootstrap support based on 1000 replicates shown at nodes. New sequences highlighted in bold. Number after host refers to individual fish number and letter refers to clone from that sample. Locality of new sequence isolates and *Kudoo thyrates* isolates indicated prior to GenBank accession number. Locality key: Au – Australia; Az – Azores; BC – British Columbia (Canada); Ch – Chile; En – England; HI – Heron Island; LI – Lizard Island; Or – Oregon (USA); SA – South Africa.

Kudoa hypoeipicardialis Blaylock, Bullard et Whipps, 2004

Total number of hosts: 7. Perciformes: Carangidae – *Caranx crysos*. Lobotidae – *Lobotes surinamensis*. Lutjanidae – *Lutjanus canipechanus*. Nomeidae – *Nomeus gronovii* (type host). Pomatomidae – *Pomatomus saltatrix*. Sciaenidae – *Pogonias cromis*. Serranidae – *Epinephelus nigritus*.

Reference: Blaylock et al. 2004.

Kudoa iwatai Egusa et Shiomitsu, 1983

Total number of hosts: 19. Beloniformes: Hemiramphidae – *Hyporhamphus gamberur*. Mugiliformes: Mugilidae – *Mugil cephalus*. Perciformes: Apogonidae – *Apogon aureus*, *A. fleurieu*. Carangidae – *Seriola quinqueradiata*. Chaetodontidae – *Chaetodon paucifasciatus*. Lethrinidae – *Lethrinus nebulosus*, *L. variegatus*. Lutjanidae – *Lutjanus erythropterus*. Moronidae – *Dicentrarchus labrax*. Oplegnathidae – *Oplegnathus fasciatus*, *O. punctatus*. Pomacentridae – *Dascyllus trimaculatus*, *Neopomacentrus miryae*. Priacanthidae – *Priacanthus hamur*. Siganidae – *Siganus rivulatus*. Sparidae – *Argyrops filamentosus*, *Pagrus major* (type host), *Sparus aurata*.

References: Paperna 1982, Egusa and Shiomitsu 1983, Egusa 1986a, Sugiyama et al. 1999, Diamant et al. 2005, Wang et al. 2005, Holzer et al. 2006.

Kudoa kenti Burger et Adlard, 2010

Total number of hosts: 4. Perciformes: Pomacentridae – *Amphiprion akindynos*, *A. melanopus* (type host), *Dischistodus perspicillatus*, *Plectroglyphidodon leucozonus*.

Reference: Burger and Adlard 2010.

Kudoa lethrini Burger, Cribb et Adlard, 2007

Total number of hosts: 2. Perciformes: Lethrinidae: *Gymnocranius audleyi* (type host), *Lethrinus harak*.

Reference: Burger et al. 2007.

Kudoa lunata Lom, Dyková et Lhotáková, 1983

Total number of hosts: 3. Pleuronectiformes: Bothidae – *Arnoglossus imperialis* (type host), *A. laterna*, *A. thori*.

Reference: Lom et al. 1983.

Kudoa megacapsula Yokoyama et Itoh, 2005

Total number of hosts: 2. Perciformes: Carangidae – *Seriola quinqueradiata*. Sphyracidae – *Sphyracna pinguis* (type host).

References: Yokoyama and Itoh 2005, Yokoyama et al. 2006.

Note: There is a possible synonym of *K. megacapsula*. *Tetrapsina decapterus* Xie et Chen, 1988 described from *Decapterus maruadsi* (Carangidae, Perciformes) has extraordinary morphological similarity with *K. megacapsula* and has all the features of *Kudoa* (see Naidjenova and Gajevskaja 1991). The genus proposed by Xie and Chen (1988) is not valid as it is based on the family Tetracapsulidae Shulman, 1959 which does not follow the International Code of Zoological Nomenclature Article 11.7.1.1, and has since been recognised as a synonym of *Kudoa* (see Lom and Dyková 2006). Until this species identity is confirmed, we will not officially associate *D. maruadsi* as a host of *K. megacapsula*.

Kudoa miniauriculata Whitaker, Kent et Sakanari, 1996

Total number of hosts: 2. Scorpaeniformes: Sebastidae – *Sebastes elongatus*, *S. paucispinis* (type host).

References: Moser et al. 1976, Heckmann and Jensen 1978, Whitaker et al. 1996, Hervio et al. 1997, Moran et al. 1999.

Kudoa nova Naidjenova, 1975

Total number of hosts: 20. Perciformes: Carangidae – *Trachurus mediterraneus ponticus*, *T. picturatus*, *T. trachurus*, *T. trecae*. Gobiidae – *Gobius niger*, *G. ophiocephalus*, *Knipowitschia longicaudata*, *Neogobius cephalaris*, *N. fluviatilis*, *N. melanostomus*, *N. platystris*, *N. ratan*, *N. syrman*, *Pomatoschistus marmoratus* (syn. *Pomatoschistus microps leopardinus* in Kovaleva et al. 1979), *Proterorhinus marmoratus*. Pomatomidae – *Pomatomus saltatrix* (type host). Scombridae – *Euthynnus alleteratus*, *Thunnus obesus*. Sparidae – *Dentex macrophthalmus*, *Pagellus acarne*.

References: Aldrin 1962, Naidjenova et al. 1975, Kovaleva et al. 1979, Campbell 2005, Gorchanok and Yurakhno 2005, Machkevsky and Gorchanok 2005.

Kudoa ovivora Swearer et Robertson, 1999

Total number of hosts: 7. Perciformes: Labridae – *Halichoeres bivittatus*, *H. garnoti*, *H. poeyi*, *Thalassoma bifasciatum* (type host). Scaridae – *Sparisoma aurofrenatum*, *S. radians*, *S. rubripinne*.

References: Swearer and Robertson 1999, Whipps et al. 2004.

Kudoa paraquadriscornis Burger et Adlard, 2010

Total number of hosts: 4. Perciformes: Carangidae – *Carangoides plagiotaenia*, *Caranx ignobilis* (type host), *C. papuensis*, *C. sexfasciatus*.

Reference: Burger and Adlard 2010.

Kudoa quadratum (Thélohan, 1895)

Total number of hosts: 10. Perciformes: Blenniidae – *Blenius gattorugine*. Callionymidae – *Callionymus lyra*. Carangidae – *Trachurus trachurus*. Labridae – *Coris julis*. Scorpaeniformes: Cottidae – *Myoxocephalus scorpius* (type host). Siluriformes: Ariidae – *Plicofollis polystaphylodon* (*Ariodes polystaphylodon* in Shulman 1988). Syngnathiformes: Syngnathidae – *Entelurus aequoreus* (syn. *Nerophis aequoreus* in Shulman 1988), *E. olquerus*, *Syngnathus acus*, *S. tenuirostris*.

References: Thélohan 1895, Parisi 1912, Meglitsch 1947, Kovaleva et al. 1979, Shulman 1988, Longshaw et al. 2004.

Kudoa sciaenae Terán, Llicán et Luque, 1990

Total number of hosts: 4. Pleuronectiformes: Sciaenidae – *Paralichthys peruianus*, *Sciaena delicosa* (type host), *S. fasciata*, *Stellifer minor*.

References: Terán et al. 1990, Oliva et al. 1992.

Kudoa shiomitsui Egusa et Shiomitsu, 1983

Total number of hosts: 2. Pleuronectiformes: Paralichthyidae – *Paralichthys olivaceus*. Tetraodontiformes: Tetraodontidae – *Takifugu rubripes* (type host).

References: Egusa and Shiomitsu 1983, Ogawa and Inouye

1997. Direct submission to GenBank of partial small subunit rDNA sequence for *P. olivaceus*.

Kudoa thalassomi Adlard, Bryant, Whipps et Kent, 2005

Total number of hosts: 18. Perciformes: See above in new material for *K. thalassomi*.

Reference: Adlard et al. 2005.

Kudoa thyrsites (Gilchrist, 1924)

Total number of hosts: 38. Beryciformes: Berycidae – *Beryx splendens*. Clupeiformes: Clupeidae – *Sardinella lemuru*, *Sardinops ocellatus*, *Sardinops sagax neopilchardus*, *Spratelloides robustus*. Engraulidae – *Engraulis australis*, *E. japonicus*. Gadiformes: Gadidae – *Theragra chalcogramma*. Merlucciidae – *Merluccius capensis*, *M. productus*. Gasterosteiformes: Aulorhynchidae – *Aulorhynchus flavidus*. Perciformes: Coryphaenidae – *Coryphaena hippurus*. Gempylidae – *Thyrsites atun* (type host). Pholidae – *Pholis ornata*. Scombridae – *Scomber japonicus*, *S. scombrus*. Trichuridae – *Lepidopus caudatus*. Pleuronectiformes: Cynoglossidae – *Pleuronectes bilineatus*. Paralichthyidae – *Paralichthys adspersus*, *P. olivaceus*. Pleuronectidae – *Atheresthes stomias*, *Eopsetta jordani*, *Hippoglossus stenolepis*, *Lepidosetta bilineata*, *Microstomus pacificus*, *Parophrys vetula*, *Platichthys stellatus*. Salmoniformes: Salmonidae – *Oncorhynchus gorbuscha*, *O. kisutch*, *O. mykiss* (syn. *Salmo gairdneri* in Kabata and Whitaker 1989), *O. tshawytscha*, *Salmo salar*, *S. trutta*. Scorpaeniformes: Cottidae – *Icelinus filamentosus*, *Leptocottus armatus*. Hexagrammidae – *Ophiodon elongatus*. Zeiformes: Zeidae – *Zeus capensis*, *Z. faber*.

References: Gilchrist 1924, Pérard 1928, Davies and Beyers 1947, Meglitsch 1947, Willis 1949, Prudhomme and Pantaleon 1959, Patashnik and Groninger 1964, Priebe 1967, Kovaleva et al. 1979, Kabata and Whitaker 1981, Patashnik et al. 1982, Harrell and Scott 1985, Kabata et al. 1986, Stehr and Whitaker 1986, Kudo et al. 1987, Kabata and Whitaker 1989, Langdon 1991, Langdon et al. 1992, Barja and Toranzo 1993, Holliman 1994, McDonald and Margolis 1995, Castro and Burgos 1996, Hervio et al. 1997, Shaw et al. 1997, Moran and Kent 1999, Whipps et al. 2003, Shukhgalter 2004, Yokoyama et al. 2004, Yokoyama and Itoh 2005, Whipps and Kent 2006, Levsen et al. 2008.

Kudoa trifolia Holzer, Blasco-Costa, Sarabev, Ovcharenko et Balbuena, 2006

Total number of hosts: 2. Mugiliformes: Mugilidae – *Liza aurata* (type host), *L. ramada*.

Reference: Holzer et al. 2006.

Kudoa unicapsula Yurakhno, Ovcharenko, Holzer, Sarabev et Balbuena, 2007

Total number of hosts: 2. Mugiliformes: Mugilidae – *Liza aurata*, *L. ramada* (type host).

Reference: Yurakhno et al. 2007.

Kudoa whippsi Burger et Adlard, 2010

Total number of hosts: 9. Perciformes: Apogonidae – *Apogon properuptus*. Pomacentridae – *Abudefduf bengalensis*, *Ab. whiteleyi*, *Acanthochromis polyacanthus*, *Amphiprion akin-*

dynos, *Am. melanopus*, *Chromis viridis*, *Neoglyphidodon melas*, *Pomacentrus chrysurus*.

Reference: Burger and Adlard 2010.

Kudoa yasunagai (Hsieh et Chen, 1984)

Total number of hosts: 7. Perciformes: Carangidae – *Seriola quinqueradiata*. Lateolabridae – *Lateolabrax japonicus* (type host). Oplegnathidae – *Oplegnathus fasciatus*. Sparidae – *Pagrus major*. Pleuronectiformes: Paralichthyidae – *Paralichthys olivaceus*. Siluriformes: Plotosidae – *Plotosus lineatus* (syn. *Plotosus anguillaris* in Cheung and Nigrelli 1990). Tetraodontiformes: Tetraodontidae – *Takifugu rubripes*.

References: Yasunaga et al. 1981, Hsieh and Chen 1984, Egusa 1986b, Cheung and Nigrelli 1990, Ogawa and Inouye 1997, Whipps et al. 2004.

Genetic intraspecific variation in kudoids among different host isolates

The trimmed SSU rDNA sequence alignment contained 109 *Kudoa* sequences from 36 species and was 1539 nucleotides long. Of the sequences that spanned the whole alignment, the sequence size varied in length from 1403 nucleotides (*K. unicapsula*) to 1512 (*K. crumena* Iversen et Van Meter, 1967). The trimmed LSU rDNA sequence alignment contained 88 *Kudoa* sequences from 20 species and was 814 nucleotides long. The sequences varied in length from 644 (*K. ovivora*) to 759 (*K. kenti*) nucleotides.

From the SSU rDNA analysis, 13 of the 36 species had DNA sequences from multiple hosts (Fig. 3). From these 13 *Kudoa* species, only *K. lethrini* (two hosts), *K. megacapsula* (two hosts), *K. paraquadrucornis* (four hosts) and *K. shiomitsui* (two hosts) have SSU sequences from all their known hosts.

In the SSU analysis, the numbers of different host families per *Kudoa* species ranged from one (*K. alliararia*, *K. gunterae*, *K. kenti*, and *K. paraquadrucornis*), up to 13 (*K. thyrsites*). Nucleotide variance between different host isolates of the same kudoid species ranged from zero (*K. paraquadrucornis* and *K. shiomitsui*) to 14 (0.9% in *K. thyrsites*, where the maximum distance was between isolates from both *Beryx splendens* Lowe and *Paralichthys olivaceus* Temminck et Schlegel compared with those from both *Merluccius capensis* Castelnau and *Thyrsites atun* (Euphrasen) nucleotides). The second largest SSU genetic variance amongst host isolates in a single *Kudoa* species was from *K. thalassomi*. Out of the eight sequences from five different host families, the range of nucleotide variance is between zero and 10 (0.7%), with the maximum occurring between isolates from the serranid host *Cephalopholis boenak* and a pomacentrid host *Neoglyphidodon melas*.

Analysis of LSU rDNA (Fig. 4) revealed that seven of the 18 *Kudoa* species have LSU rDNA sequences from more than one host and of these seven, only *K. kenti* (four hosts), *K. paraquadrucornis* (four hosts), and *K. thalas-*

Fig. 5. Principal component analysis with 95% ellipses of morphometric data from apical measurements of *Kudoa thalassomi*. Open squares – 6-polar capsule morphotype from *Abudedefduf bengalensis*; plus signs – 7-polar capsule morphotype from *Abudedefduf bengalensis*; filled squares – 6-polar capsule morphotype from *Thalassoma lunare*; crosses – 7-polar capsule morphotype from *Thalassoma lunare*.

somi (18 hosts) have LSU sequences from all their known hosts.

The number of different host families per *Kudoa* species with LSU rDNA sequences ranged from one (*K. gunterae*, *K. kenti*, and *K. paraquadricornis*) to 10 (*K. thyrsites*). Nucleotide differences between isolates from different hosts ranged from zero (*K. paraquadricornis*) to 59 (9.2% in *K. thyrsites*, where the maximum distance was between isolates from the hosts *Coryphaena hippurus* Linnaeus and *Paralichthys olivaceus*). The second largest LSU genetic variance amongst isolates from different hosts in a single *Kudoa* species was again demonstrated by *K. thalassomi*. Out of the 24 sequences of *K. thalassomi* from the six different host families, the nucleotide variance ranged from one (0.1%) to 14 (2.1%), while the maximum difference was between isolates from a scarid host (*Scarus flavipectoralis*) with isolates from two different chaetodontid hosts (*Chaetodon unimaculatus* and *Chelmon rostratus*) and one from a pomacentrid host (*Neoglyphidodon melas*).

It is noteworthy that there was significant LSU sequence variance between *K. thalassomi* isolates collected from different individuals of the same host species. For example, isolates from two individuals of *Amblyglyphidodon curacao* showed 11 nucleotide differences (1.6%). Genetic variation also occurred at other levels, e.g. two individual kudoid LSU genotypes (single genotypes isolated and amplified using bacterial cloning) from a single *Amphiprion akindynos* individual showed 6 nucleotide

differences (0.9%), while four sequences from isolates derived from three individuals of *Chaetodon unimaculatus* differed by 4–11 nucleotides (0.6–1.2%) and sequences from isolates derived from each of two *Thalassoma lunare* (the type host) individuals showed 4 nucleotide differences (0.6%).

DISCUSSION

Kudoa thalassomi is one of four *Kudoa* species described to date represented by a dominant 6-polar capsule spore morphotype and that infect the somatic musculature of their host fish. One of the other three species, *Kudoa neothunni* infects yellowfin tuna from Japan and is distinguished from *K. thalassomi* by having smaller polar capsules. The other two of the three species, *K. grammatorcyni* and *K. scomberomori*, are sympatric with *K. thalassomi* i.e. they occur on the Great Barrier Reef, Australia, but are smaller in spore size (Table 1) and are readily distinguished in genetic analyses (Figs. 3, 4). Interestingly, *K. thalassomi* is closer in genetic distance to two species that infect brain tissue, *K. lethrini*, a species dominated by a 7-polar capsule morphotype also from the Great Barrier Reef, and *K. neurophila* (Grossel, Dyková, Handlinger et Munday, 2003), a species dominated by a 5-polar capsule morphotype from Tasmania in southern Australia (having 21 nucleotides and 1.5% difference in SSU to both species) (Grossel et al. 2003). *Kudoa thalassomi* differs in SSU sequence from *K. grammatorcyni* and *K. scomberomori* by 2.4% and 2.5%, respectively. What drives such a genetic relationship that places *K. thalassomi* closer to brain-infecting species is unclear. *Kudoa lethrini* and *K. neurophila* infect different host tissues, are not more morphologically similar and do not infect either genetically or ecologically similar hosts. We suspect that with more comprehensive taxon sampling the basis for such relationships will become clear.

Kudoa thalassomi also differs from related muscle-infecting species, *K. grammatorcyni* and *K. scomberomori*, in having a low host specificity. From 214 fish species the authors sampled on the Great Barrier Reef, *K. grammatorcyni* has been found only in *Grammatorcynus bicarinatus* Quoy et Gaimard (see Adlard et al. 2005) and *G. bilineatus* (Rüppell) (unpublished data), while *K. scomberomori* has been found only in *Scomberomorus commerson* Lacépède (see Adlard et al. 2005). *Kudoa thalassomi* has now been found in 18 host species from six different host families (see Fig. 4 and new material detailed in the Results).

Kudoa thalassomi has now been recorded from the highest number of host species for any kudoid species for which the specific identity has been confirmed through DNA sequence analysis (LSU rDNA). In comparison, *K. thyrsites* has DNA sequences matching a broader range of host species (14 kudoid isolates having SSU rDNA sequences, and 11 isolates with LSU rDNA sequences)

and geographic distribution with representatives from 13 different host families within eight host orders (see Figs. 3, 4); however, this increased host diversity and geographic range also corresponds to a higher maximum sequence difference in *K. thyrsites* isolates, especially in LSU rDNA which has been shown to be a superior indicator of species differentiation than SSU (see Burger and Adlard 2010). In the LSU, *Kudoa thalassomi* has 2.1% maximum sequence variation between isolates, while *K. thyrsites* has a 9.2% variation. The total number of *K. thyrsites* host species for which there are DNA sequences (14) is less than half (37%) of the total number of recorded hosts (38) for that species. This suggests that the maximum sequence variation in *K. thyrsites* isolates may be higher than that recorded to date. In comparison, there are DNA sequences (LSU) of *K. thalassomi* for all of the currently recorded hosts for that parasite.

There have been many records of *K. thyrsites* since it was first described by Gilchrist in 1924 which, when combined, report a large morphological variance for spores of this species. For example, the smallest average width of a *K. thyrsites* isolate was 12.0 μm (Gilchrist 1924) from *Thyrsites atun* compared to the largest of 16.7 μm from *Merluccius productus* (Ayres) (see Kabata and Whitaker 1981, Whipps and Kent 2006). Smaller, but significant morphometric differences have also been noted between host isolates of *K. hypoepicardialis* with mean spore width of 10.1 μm (range 9.3–11.2 μm) from *Nomeus grovonii* (Gmelin), and 7.6 μm (range 6.5–8.4 μm) from *Pogonias cromis* (Linnaeus) (see Blaylock et al. 2004). Our data show evidence for host-induced variation in spore valve size between *K. thalassomi* isolates from *Abudefduf bengalensis* and those from *Thalassoma lunare* (average spore width of the 6-polar capsule morphotype was 13.7 μm (range 12.4–14.9 μm) for spores from *A. bengalensis* and 13.2 μm (range 12.3–14.9 μm) for spores from *T. lunare*). We suspect that a small proportion of the morphological variation reported here and the original description of *K. thalassomi* ex *T. lunare* (mean spore width 10.7 μm , range 9.5–11.8 μm) may have been the result of post-collection treatment of specimens (e.g. number of times frozen/thawed), but clearly there is a degree of variation in spore dimensions displayed by even the same genetic species, for example the previously mentioned *K. hypoepicardialis* isolates all have identical SSU sequences (Blaylock et al. 2004) (see Fig. 3). Given that kudoid species are now known to display a degree of morphometric plasticity (see Blaylock et al. 2004, Whipps and Diggles 2006, Whipps and Kent 2006, Burger and Adlard 2010), it is even more critical that an independent character set such as DNA sequence be employed as a datum to help establish species boundaries. Nonetheless, morphology still provides useful characters for defining species thus we would favour a whole evidence approach for identification.

Fig. 6. Frequency distribution of recorded host specificity of *Kudoa* species.

Prior to the use of DNA, all *Kudoa* species with a stellate spore shape and one polar capsule larger than the other three were likely to be assigned to *K. thyrsites*, with the only recorded exceptions being *K. cruciformum* (Matsumoto, 1954) and *K. mirabilis* Naidjenova et Gajevskaja, 1991. With the benefit of DNA data it now appears that *K. thyrsites* may represent a species complex (Moran et al. 1999, Whipps and Kent 2006) due largely to the significant genetic variance among isolates from different hosts and to the broad geographic distribution of this species. How many species are represented by *K. thyrsites* is currently uncertain and will remain so until morphological or biological characters are discovered that map, with confidence, to genetic profiles.

Kudoa cruciformum and *K. lateolabracis* Yokoyama, Whipps, Kent, Mizuno et Kawakami, 2004 are clearly species that are closely related morphologically but are considered to be distinct (Yokoyama et al. 2004). No DNA data are yet available for *K. cruciformum*. In SSU rDNA *K. lateolabracis* is 23–39 nucleotides (1.9–2.0%) different to sequences from *K. thyrsites* (see Yokoyama et al. 2004).

Kudoa mirabilis seems distinct from all other species based on its morphology (it has a much more significant difference between the sizes of the large polar capsule compared to the three small polar capsules) and from the distinctive pathology associated with the infection. Naidjenova and Gajevskaja (1991) describe macroscopic cysts (3–5 mm diameter; occurring in clusters) that cause blisters filled with spores on the body surface and inside the body. This pathological presentation has not been seen with any other stellate, unequal polar capsule kudoid.

Since the use of DNA characterisation, it has been possible to distinguish unambiguously the 4-polar capsule, stellate kudoids *K. gunterae*, *K. lateolabracis*, *K. minithyrsites* Whipps, Adlard, Bryant, Lester, Findlay et Kent, 2003, and *K. whipsi* from *K. thyrsites* (see Figs.

3 and 4). In addition, DNA has also assisted in resolving confusion with other species identities. For example, the close genetic similarity between the *K. iwatai* sequences published by Diamant et al. (2005) and *K. lutjanus* from Wang et al. (2005) coupled with spore shape and tissue tropism led to the latter species being considered a junior synonym of *K. iwatai* (see Holzer et al. 2006). Another example is the sequences of *K. rosenbuschi* published by Abollo et al. (2005) and *Kudoa* sp. published by Pascual and Abollo (2008) which had high sequence similarity with that of *K. alliaria* published by Whipps and Diggles (2006). The infections that these sequences represent have now been recognised as referable to *K. alliaria* (see Burger and Adlard 2010) with further evidence from the identical pathology that they cause in the host.

An example of a possible synonym that could be determined using DNA is that of two cardiac tissue-infecting species *K. pagrusi* Al Quraishy, Koura, Abdel-Baki, Bashtar, El Deed, Al Rasheid et Abdel Ghaffar, 2008, described from *Pagrus pagrus* in the Red Sea and *K. shiomitsui* (see Egusa and Shiomitsu 1983) described from *Takifugu rubripes* from Japan. The spores are similar in size and shape and the cyst shape and location in the host tissues are identical. Also, it has previously been shown that *K. iwatai* has been found in both Japan and in the Red Sea (Diamant et al. 2005), reducing any emphasis for species distinction based on large geographic separation. It appears that the proposers of *K. pagrusi* were unaware of the existence of *K. shiomitsui*, as they did not include any reference to this species in their publication. Also, the authors may have misidentified the fish host because *Pagrus pagrus* does not occur in the Red Sea; instead it is found in the Atlantic Ocean and the Mediterranean Sea (<http://www.fishbase.org/>). A SSU rDNA sequence of the infection from the sparid fish from Al Quraishy et al. 2008 should be able to determine its relationship with reference to *K. shiomitsui* which has two SSU sequences available (see Fig. 3).

The distinction between *K. clupeiidae* from *Brevoortia tyrannus* (Latrobe) published by Webb et al. (2005) and *K. funduli* from *Fundulus heteroclitus* (Linnaeus) published by Akaishi et al. (2004) may be invalid because SSU rDNA sequences are identical (see Fig. 3). Meglitsch (1948) differentiated between these two morphologically similar *Kudoa* species on several subtle shape and size differences and that *K. funduli* was found also on the fish fins rather than only within somatic muscle. The lack of differentiation between these two species in the SSU rDNA analysis does not include the four indels that occur in the alignment as differences between the two sequences. The lack of informative characters is accentuated because the *K. funduli* sequence is much shorter (739 nucleotides long in the alignment from this study) than that of *K. clupeiidae* (1394 nucleotides long in the alignment from this study) and does not span across one of the more highly variable

regions of the SSU rDNA fragment at the 5' end of the gene. Therefore, until more sequences are generated for these two species we consider that they should be maintained.

The examples given above demonstrate that having multiple host records for a kudoid species should not, of itself, be an indicator of broad host specificity. Unless such records include DNA sequences from different hosts such a conclusion may be unwarranted. By increasing the number of parasite/host isolates represented in the DNA database, the more certainty there will be in the host range of species in the Myxosporea. Just over half of the total number of *Kudoa* species with multiple hosts listed in the results section have been sequenced from 50% or more of their recorded hosts (14 of 27); and less than a quarter of the total number of species have DNA sequences representing their entire host range (6 of 27: *K. lethrini*, *K. kenti*, *K. megacapsula*, *K. paraquadricornis*, *K. shiomitsui*, and *K. thalassomi*). Although the numbers are relatively low, patterns in host specificity are slowly becoming apparent in the Kudoidae.

The specific mechanisms that drive host specificity of kudoids are unknown. In the Bivalvulida, actinospores of *Myxobolus cerebralis* Hofer, 1903 respond positively to signals in the mucus of a range of fish, but infection only progresses in susceptible hosts (Kallert et al. 2009). Yokoyama et al. (2006) showed that *Myxobolus arcticus* Pugachev et Khokhlov, 1979 actinospores responded quickly and non-specifically to fish mucus, but *Thelohanellus hovorkai* Akhmerov, 1960 actinospores showed a slower selective response to mucus from the susceptible carp host. Clearly, development in any particular host relies on a combination of cues to enhance the encounter of infective stages with hosts, followed by the ability of parasites to establish infections regardless of host defensive mechanisms.

At higher taxonomic levels the specificity of kudoid species does not appear to show any broad trends. Taxonomic relatedness of hosts is not significantly correlated with genetic relatedness of species in the Myxosporea for either multivalvulids (see Burger et al. 2007) or for bivalvulids within the Ceratomyxidae (see Gunter et al. 2009), Chloromyxidae (see Holzer et al. 2004), Myxidiidae (see Holzer et al. 2004, Fiala 2006), Myxobolidae (see Andree et al. 1999), and Sphaerosporidae (see Holzer et al. 2004).

At a species level, the majority of kudoids have only been reported from a single host species, but a significant proportion display a broader host range. Furthermore, the host range of a species is often more a function of research effort rather than a true representation of its range. Only a few myxosporean genera have been studied with sufficient intensity to have confidence in specificity trends, with *Ceratomyxa* and *Myxobolus* showing relatively high levels of host specificity (Molnár 1994,

Gunter et al. 2009). Prior to this study, *K. thalassomi* was reported only from *Thalassoma lunare*, but now from the data we present in this study it is obvious that the host specificity of *K. thalassomi* is broad. This is a clear demonstration that any interpretation of host specificity cannot be made from serendipitous records of presence in a particular host, but rather, through a focused study using combined morphological and molecular characters to allow unambiguous identification of parasites from multiple hosts. Regardless of the current interest in myxosporean parasites, it will likely take some years to increase and validate the taxonomic dataset to a level where further trends in host specificity can be predicted with any confidence for these parasites. Results from this study reveal that myxosporeans in general and kudoids in particular, have adopted strategies that result in various levels of host specificity. Nonetheless, identifying the host range of parasites can be of enormous value for predicting the threat of introduction of a potential pathogen and for managing an existing disease.

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