### Original: English (EN)

November-2 December 2021 & May-June 2022

# Report of the WOAH ad hoc Group on susceptibility of mollusc species to infection with OIE listed diseases



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## 1. Meetings

This report covers the work of the WOAH *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases (the *ad hoc* Group) who met virtually between November and December 2021 and between May and June 2022.

The list of participants and the Terms of Reference are presented in Annex I and Annex II, respectively.

# 2. Methodology

The *ad hoc* Group applied criteria, as outlined in Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen of the WOAH *Aquatic Animal Health Code* (the *Aquatic Code*), to potential host species in order to determine susceptibility to infection with *Marteilia refringens*. The assessments were conducted using a three-stage approach. Details of the three-stage approach, including additional considerations, are described below:

# 2.1. Stage 1: Criteria to determine whether the route of transmission is consistent with natural pathways for the infection (as described in Article 1.5.4.):

Table 1 describes the route of transmission for infection with *Marteilia refringens* used by the *ad hoc* Group when applying Stage 1 to assess susceptibility to infection with *Marteilia refringens*.

Table 1: Route of transmission for infection with Marteilia refringens

	Route of Transmission	Considerations						
1.	Natural exposure included situations where infection had occurred without experimental intervention (e.g. infection in wild or farmed populations).	Non-invasive experiments were considered for copepods and for <i>Mytilus galloprovincialis</i> (Comps & Joly, 1980).						
OR								
2.	Non-invasive experimental procedures: e.g. cohabitation with infected hosts or faeces of infected hosts (Carrasco <i>et al.</i> , 2008b); or infection by immersion in seawater enriched with a suspension of parasites (Comps & Joly, 1980).							

# 2.2. Stage 2: Criteria to determine whether the pathogenic agent has been adequately identified (as described in Article 1.5.5.):

Table 2 describes the pathogen identification methods used by the *ad hoc* Group when applying Stage 2 to susceptibility to infection with *Marteilia refringens*, as well as some considerations.

Table 2: Pathogen Identification for infection with Marteilia refringens

Pathogen Identification ( <i>Marteilia refringens</i> )	Pathogen Identification (at Type M or Type O level )*	Considerations
Molecular sequence information for Internal transcribed spacer 1 (ITS 1) (Le Roux <i>et al.</i> , 2001) or intergenic spacer (IGS) (Lopez-Flores <i>et al.</i> , 2004) regions	Molecular sequence information for ITS 1 (Le Roux <i>et al.</i> , 2001) or intergenic spaces (IGS) (Lopez-Flores <i>et al.</i> , 2004) regions OR	Molecular sequence information within the 18S sequence generally does not allow differentiation between Type O, Type M or Marteilia cochillia
OR PCR-RFLP (as described in Le Roux <i>et al.</i> , 2001)	PCR-RFLP (as described in Le Roux <i>et al.</i> , 2001) which distinguishes between Type O and	Differences between Type O and Type M are based on the ITS 1 and consistent with the differences defined in IGS
OR  Multiplex TaqMan Assay to detect Marteilia refringens (Carrasco et al., 2017)  OR  Observed parasite and morphology from histology or cytology that was later characterised by linked molecular information from other studies.	Type M OR Multiplex TaqMan Assay to detect Marteilia refringens and distinguishes between types Type O and M (Carrasco et al., 2017) OR Observed parasite and morphology from histology or cytology that was later characterised by linked molecular information from other studies (Type O and Type M).	Molecular data should be associated with microscopical examination wherever possible to confirm the presence of the pathogen.  ISH is currently not sufficiently specific to resolve pathogen identity at the species and type levels.  For early studies without molecular information, corroborating evidence from later studies was also considered*.

<sup>\*</sup> In locations where co-occurrences of type O and type M have been reported, the *ad hoc* Group could not necessarily link stage 3 criteria demonstrating the presence of a pathogenic agent that constitutes an infection, to a single genetic type. When a study is not strong enough (limited sample size or representativeness between geographic region, time and host species), the *ad hoc* Group could not exclude the presence of the alternate type of *M. refringens*. In contrast, the *ad hoc* Group could attribute infection to a particular type and rule out co-infection when molecular methods that maximise chance to detect co-infection including multiplex tagman assay and cloning prior PCR-RFLP or sequencing were used.

# 2.3. Stage 3: Criteria to determine whether the evidence indicates that presence of the pathogenic agent constitutes an infection (as described in Article 1.5.6.):

Evidence to support criterion A alone was sufficient to determine infection. In the absence of evidence to meet criterion A, satisfying at least two of criteria B, C or D were required to determine infection. Criteria A to D are presented below:

- A. The pathogenic agent is multiplying in the host, or developing stages of the pathogenic agent are present in or on the host<sup>1</sup>:
- B. Viable pathogenic agent is isolated from the proposed susceptible species, or infectivity is demonstrated by way of transmission to naïve individuals;
- C. Clinical or pathological changes are associated with the infection;
- D. The specific location of the pathogen corresponds with the expected target tissues.

<sup>&</sup>lt;sup>1</sup> For the purposes of the assessments for susceptibility to infection with *Marteilia refringens*, replication 'on the host' was not considered to apply.

Table 3 describes the evidence of infection with *Marteilia refringens*, Type O and Type M, used by the *ad hoc* Group when applying Stage 3 to susceptibility to infection with *Marteilia refringens*, as well as some considerations.

Table 3: Evidence of infection with Marteilia refringens

		Evidence o	of infection	
	A: Replication	B: Viability / Infectivity	C** Pathology / Clinical signs	D***: Location
1.	Presence of the mature stage (equals presence of tertiary cells) of the parasite demonstrated by:  a) Histopathology	Transmission via either co-habitation or faeces exposure to copepods  OR      Demonstration of viability of cells isolated	Mortality <sup>2</sup> OR      Macroscopic lesions such as discolouration of tissue (pale digestive gland)	Parasites in the epithelia of digestive gland tissue  OR      Atypical location within hemolymph, or connective tissue of
	OR b) Cytology (usually digestive gland imprints)	from tissues and of spores in faeces by:  a) Vital stains  OR	OR 3. Rapid loss of condition OR	different organs including the gills, and the mantle <sup>3</sup> OR
<b>O</b> I 2.	OR c) TEM  R  For copepods, different parasite stages or the presence of many parasite cells.	b) Successful infection of copepods.	4. Microscopic lesions such as localised haemocyte infiltration in connective tissues around the digestive gland	3. For copepods, parasites located in the gonad and/or the digestive tract.

<sup>\*\*</sup> Pathology/Clinical signs may be non-specific, variable and include some or all of the characteristics listed.

\*\*\*As demonstrated by histology or *in-situ* hybridisation (ISH) or positive PCR from the digestive gland tissue.

#### 3. Results

The ad hoc Group agreed that only three of the six species currently included in Article 11.4.2. as susceptible to infection with Marteilia refringens, blue mussel (Mytilus edulis), European flat oyster (Ostrea edulis) and the Mediterranean mussel (Mytilus galloprovincialis) met the criteria for listing as susceptible to infection with Marteilia refringens in accordance with Chapter 1.5. of the Aquatic Code and were proposed to remain in Article 11.4.2. Three species, the Australian mud oyster (Ostrea angasi), Argentinean oyster (Ostrea puelchana) and the Chilean flat oyster (Ostrea chilensis) did not meet the criteria for listing as a susceptible species and were proposed to be deleted from Article 11.4.2.

Five additional species were found to meet the criteria for listing as susceptible species to infection with *Marteilia refringens*. Dwarf oyster (*Ostrea stentina*), European razor clam (*Solen marginatus*), golden mussel (*Xenostrobus securis*), striped venus (*Chamelea gallina*) and a copepod (*Paracartia grani*), were proposed to be included in Article 11.4.2.

Two Ostrea species, Chilean flat oyster (Ostrea chilensis) and Japanese flat oyster (Ostrea denselamellosa) and a copepod (Paracartia latisetosa) were assessed as having incomplete evidence of susceptibility and were proposed to be included of Section 2.2.2. of Chapter 2.4.4., Infection with Marteilia refringens, of the Aquatic Manual.

Pathogen-specific positive PCR results had been reported in the following seven species, Cortez oyster (*Crassostrea corteziensis*), Grooved carpet shell (*Ruditapes decussatus*), Pacific cupped oyster (*Magallana gigas* also known as *Crassostrea gigas*) and zooplankton (*Acartia discaudata, Centropages typicus, Euterpina acutifrons, Penilia avirostris*) as well as in unidentified copepods of the genus *Oithona*, but an active infection had not been

<sup>&</sup>lt;sup>2</sup> It is sometimes difficult to correlate the presence of the pathogen with mortality. In this case, mortality alone was not sufficient when other pathogens were documented to be present.

<sup>&</sup>lt;sup>3</sup> To date the atypical location in the connective tissues has mostly been reported in mussels (WOAH Reference laboratory information).

demonstrated. These species were proposed to be included in the second paragraph of Section 2.2.2. of Chapte 2.4.4., Infection with *Marteilia refringens*, of the *Aquatic Manual*.

#### 4. Assessments

Species were determined to be susceptible based on the combination of assessment outcomes as outlined in Article 1.5.7.

Table 4 describes the different scores and outcomes of the assessments undertaken by the ad hoc Group.

**Table 4: Scores and Outcome of assessments** 

Score	Outcome
1	Species assessed as susceptible (as described in Article 1.5.7.). These species were proposed for inclusion in Article 11.4.2. of Chapter 11.4., Infection with <i>Marteilia refringens</i> , of the <i>Aquatic Code</i> and Section 2.2.1. of Chapter 2.4.4., Infection with <i>Marteilia refringens</i> , of the <i>Manual of Diagnostic Tests for Aquatic Animals</i> (the <i>Aquatic Manual</i> ).
2	Species assessed as having incomplete evidence for susceptibility (as described in Article 1.5.8.) were proposed for inclusion in Section 2.2.2., Species with incomplete evidence for susceptibility of Chapter 2.4.4., Infection with <i>Marteilia refringens</i> , of the <i>Aquatic Manual</i> .
3	Species assessed as not meeting the criteria or for which there was unresolved or conflicting information. These species were not proposed for inclusion in either the <i>Aquatic Code</i> or the <i>Aquatic Manual</i> .
	The exceptions were species where pathogen-specific positive PCR results had been reported but an active infection had not been demonstrated. These species were proposed for inclusion in the second paragraph in Section 2.2.2. Species with incomplete evidence for susceptibility of Chapter 2.4.4., Infection with <i>Marteilia refringens</i> , of the <i>Aquatic Manual</i> .
4	Species assessed as non-susceptible.
NS	Species not scored due to insufficient or irrelevant information.

Table 5 summarises the assessments for host susceptibility to infection with *Marteilia refringens* together with the outcomes and relevant references.

Table 5: Assessments for infection with Marteilia refringens

Family	Scientific name	Common name	Stage 1: Route of transmission	Stage 2: Pathogen identification	Evi		ge 3: of infect	ion	Outcome <i>M. refringens</i> – Paper	Outcome Type M		Outcome Type O		References
					Α	В	С	D		Paper	Overall	Paper	Overall	
						Scor	re 1							
Bivalves														
Ostreidae	Ostrea edulis	European flat oyster	N	IGS and ITS 1 PCR with ITS 1 sequencing	YES	ND	YES	YES	1	l <sup>5</sup>		1		Lopez-San Martin <i>et al</i> ., 2015
			N	NO (Histology <sup>6</sup> )	YES	ND	ND	YES	1	NS	NS NS 3	NS		Audemard <i>et al</i> ., 2001
			N	NO (Histology and cytology <sup>6</sup> )	YES	YES	ND	YES	1	NS		NS	1	Carrasco <i>et al</i> ., 2008b
			N	ITS 1 PCR, RFLP ITS 1 sequencing	ND	ND	ND	YES	2	3			'	Novoa <i>et al</i> ., 2005
			N	ITS 1 PCR, RFLP ITS 1 sequencing	ND	ND	ND	YES	2	3		3		Le Roux <i>et al.</i> , 2001
Ostreidae	Ostrea stentina	Dwarf oyster	N	IGS and ITS 1 PCR, RFLP, ITS 1 and IGS sequencing	YES	ND	l <sup>7</sup>	YES	1	NS	3	1	1	Elgharsalli <i>et al.</i> , 2013
			N	IGS and ITS 1 PCR, only ITS 1 sequencing	YES	ND	YES	YES	1	3		3		Lopez-SanMartin et al., 2015
Mytilidae	Mytilus edulis	Blue mussel	N	ITS1 RFLP, IGS PCR, sequencing and Histology	YES	ND	YES	YES	1	1	1	NS	NS	Bøgwald <i>et al</i> ., 2022
Mytilidae	Mytilus galloprovincialis	Mediterranean mussel	N	ITS 1 PCR, IGS, RFLP and sequencing	YES	ND	ND	YES	1	3		3		Arzul <i>et al</i> ., 2014
			N	IGS, ITS 1 PCR and sequencing	YES	ND	NO	YES	1	1	1 1	NS	3	Gombac <i>et al</i> ., 2014
			N	IGS PCR and sequencing	YES	ND	ND	YES	1	NS		NS		Carrasco et al., 2007b
Mytilidae	Xenostrobus securis	Golden mussel	N	IGS and ITS 1 PCR and sequencing	YES	ND	ND	YES	1	3	3	NS	NS	Pascual et al., 2010

Family	Scientific name	Common name	Stage 1: Route of transmission	Stage 2: Pathogen identification	Ev			Stage 3: nce of infection		Outcome Type M		Outcome Type O		References
					Α	В	С	D		Paper	Overall	Paper	Overall	
Solenidae	Solen marginatus	European razor clam	N	IGS PCR and sequencing	YES	ND	ND	YES	1	3	3	NS	NS	Lopez-Flores <i>et al</i> ., 2008a
			N	NO8 (Histology)	YES	ND	ND	YES	NS	NS		NS		Lopez & Darriba, 2006
Veneridae	Chamelea gallina	Striped venus	N	IGS sequence, Histology ISH	YES	ND	l <sup>9</sup>	YES	1	NS	NS	3	3	Lopez-Flores <i>et al.</i> , 2008b
Crustacea														
Acartiidae	Paracartia grani	No common name	N, E	PCR ITS 1, nested PCR IGS and sequencing	YES	J <sup>10</sup>	YES	YES	1	NS	NS	NS	NS	Boyer <i>et al.</i> , 2013
			E	Histology, ISH and TEM <sup>6</sup>	YES	ND	ND	YES	1	NS		NS		Carrasco et al., 2008b
						Scor	re 2							
Bivalves														
Ostreidae	Ostrea chilensis	Chilean flat oyster	N	Histology <sup>6</sup>	YES	ND	I <sup>11</sup>	YES	1	NS	NS	NS	NS	Grizel <i>et al.</i> , 1983
Ostreidae	Ostrea denselamellosa	Japanese flat oyster	N	Histology <sup>6</sup>	ND	ND	I <sup>12</sup>	YES	2	NS	NS	NS	NS	Martin, 1993
Crustacea														
Acartiidae	Paracartia latisetosa	No common name	N	PCR IGS and sequencing	YES	ND	ND	YES	1	NS	NS	NS	NS	Arzul <i>et al.</i> , 2014
						Scor	re 3							
Ostreidae	<i>Magallana</i> <i>gigas</i> also known as	Pacific cupped oyster	N	nested PCR IGS and sequencing	ND	ND	ND <sup>13</sup>	YES	3	314	3	NS	NS	Grijalva-Chon <i>et al.</i> , 2015
	Crassostrea gigas		N	NO (Histology)	NO	NO	YES	YES	NS	NS	NS	NS	NS	Cahour, 1979
Ostreidae	Crassostrea corteziensis	Cortez oyster	N	nested PCR IGS and sequencing	ND	ND	ND <sup>13</sup>	YES	3	NS	NS	3 <sup>14</sup>	3	Grijalva-Chon <i>et al.</i> , 2015
Veneridae	Ruditapes decussatus	Grooved carpet shell	N	ITS 1 PCR and nested PCR IGS and sequencing	NO	ND	NO	YES	3	3 <sup>14</sup>	3 <sup>14</sup>	NS	NS	Boyer <i>et al.</i> , 2013

Family	Scientific name	Common name	Stage 1: Route of transmission	Stage 2: Pathogen identification	Ev		ge 3: of infect	ion	Outcome Outcome M. refringens Type M - Paper			Outcome Type O		References	
					Α	В	С	D		Paper	Overall	Paper	Overall		
Crustacea									'					•	
Acartiidae	Acartia discaudata	No common name	N	PCR targeting IGS and sequencing	ND	ND	ND	ND	3	NS	NS	NS	NS	Carrasco <i>et al.</i> , 2007b	
Centropagidae	Centropages typicus	No common name	N	ITS 1 PCR, IGS, RFLP and sequencing	ND	ND	ND	NO	3	NS	NS	NS	NS	Arzul <i>et al</i> ., 2014	
Achidiidae	Euterpina acutifrons	No common	N	NO (PCR 18S with SS2/SAS1 primers <sup>15</sup> )	ND	ND	ND	NO	NS	NS	NS	NS	NS	NS	Audemard et al., 2002
		name	N	PCR targeting IGS and sequencing	ND	ND	ND	ND	NS <sup>16</sup>	NS		NS	NS		Carrasco et al., 2007b
Oithonidae	Oithona sp. (FRANCE)	No common name	N	PCR targeting IGS. ISH negative	ND	ND	ND	NO	3	NS	NS	NS	NS	Arzul <i>et al.</i> , 2014	
Oithonidae	Oithona sp. (SPAIN)	No common name	N	PCR targeting IGS and sequencing	ND	ND	ND	ND	3	NS	NS	NS	NS	Carrasco et al., 2007b	
Sididae	Penilia avirostris	No common name	N	PCR targeting ITS and IGS. RFLP	ND	ND	ND	NO	3	NS	NS	NS	NS	Arzul <i>et al</i> ., 2014	
						Score	e NS								
Bivalves															
Ostreidae	Ostrea angasi	Australian mud oyster	N	NO (Histology and cytology <sup>6</sup> )	ND	ND	<sup>17</sup>	ND	NS	NS	NS	NS	NS	Bougrier <i>et al.</i> , 1986	
Ostreidae	Ostrea puelchana	Argentinean oyster	N	NO (Histology)	ND	ND	ND	ND	NS	NS	NS	NS	NS	Pascual <i>et al</i> ., 1991	
Ostreidae	Saccostrea cuccullata	Hooded oyster	N	NO (Histology)	NO	ND	ND	YES	NS	NS	NS	NS	NS	Comps, 1976	
Ostreidae	Crassostrea viriginica	American cupped oyster	N	NO (Histology and TEM)	YES	NO	ND	YES	NS	NS	NS	NS	NS	Renault <i>et al.</i> , 1995	
Cardiidae	Cerastoderma edule	Common edible cockle	N	NO (Histology and TEM)	YES	ND	ND	YES	NS	NS	NS	NS	NS	Comps <i>et al.,</i> 1975	
			N	NO (Histology)	YES	ND	ND	YES	NS	NS		NS		Poder <i>et al.</i> ,1983	
Veneridae	Ruditapes philippinarum	Japanese carpet shell	N	NO (Histology)	YES	ND	NO	YES	NS	NS	NS	NS	NS	Itoh <i>et al</i> ., 2005	

Family	Scientific name	Common name	Stage 1: Route of transmission	Stage 2: Pathogen identification	Ev	Stage 3: Evidence of infection		ion	Outcome <i>M. refringens</i> – Paper	Outcome Type M		Outcome Type O		References
					Α	В	С	D		Paper	Overall	Paper	Overall	
Veneridae	Polititapes rhomboides	Banded carpet shell	N	NO (Histology)	YES	ND	ND	YES	NS	NS	NS	NS	NS	Poder <i>et al.,</i> 1983
Veneridae	Venerupis corrugata	Corrugated venus	N	NO (Histology)	YES	ND	ND	YES	NS	NS	NS	NS	NS	Poder <i>et al.,</i> 1983
Pharidae	Ensis minor	Clamdog	N	NO (Histology)	YES	ND	ND	YES	NS	NS	NS	NS	NS	Ceschia <i>et al.,</i> 2001
Semelidae	Scrobicularia plana	Peppery furrow	N	NO <sup>18</sup> (Histology and TEM)	YES	ND	ND	YES	NS	NS	NS	NS	NS	Comps, 1983
Pectinidae	Argopecten gibbus	Calico scallop	N	NO (Histology)	YES	ND	YES	YES	NS	NS	NS	NS	NS	Moyer <i>et al.</i> , 1993
Cardiidae	Tridacna maxima	Elongate giant clam	N	NO (Histology and TEM)	NO	ND	NO	YES	NS	NS	NS	NS	NS	Norton <i>et al</i> ., 1993
Semelidae	Abra segmentum	No common name	N	N (PCR 18S with SS2/SAS1 primers <sup>15</sup> )	ND	ND	ND	ND	NS	NS	NS	NS	NS	Audemard <i>et al.</i> , 2002
Crustacea	•													
Acartiidae	Acartia clausi		N	PCR targeting IGS and sequencing	ND	ND	ND	ND	NS	NS	NS	NS	NS	Carrasco et al., 2007b
Acartiidae	Acartia italica	No common name	N	PCR targeting IGS and sequencing	ND	ND	ND	ND	NS	NS	NS	NS	NS	Carrasco et al., 2007b
Canuellidae	Canuella perplexa	No common name	N	NO (PCR 18S with SS2/SAS1 primers <sup>15</sup> )	ND	ND	ND	ND	NS	NS	NS	NS	NS	Audemard <i>et al.</i> , 2002
Cladocera	Evadne sp.	No common name	N	PCR targeting IGS. ISH negative	ND	ND	ND	NO	NS	NS	NS	NS	NS	Arzul et al., 2014
Oithonidae	Oithona sp. (SPAIN)	No common name	N	PCR targeting ITS and IGS. RFLP	ND	ND	ND	ND	NS	NS	NS	NS	NS	Carrasco et al., 2007a
Order: Cyclopoida	ND	No common name	N	N (PCR 18S with SS2/SAS1 primers <sup>15</sup> )	ND	ND	ND	ND	NS	NS	NS	NS	NS	Audemard <i>et al.</i> , 2002
Order: Harpacticoida	ND	No common name	N	PCR targeting ITS and IGS. RFLP	ND	ND	ND	ND	NS	NS	NS	NS	NS	Carrasco et al., 2007a

Family	Scientific name	Common name	Stage 1: Route of transmission	Stage 2: Pathogen identification	Ev	Stage 3: Evidence of infection		Outcome <i>M. refringens</i> – Paper	Outcome Type M		Outcome Type O		References	
					Α	В	С	D		Paper	Overall	Paper	Overall	
Order: Decapoda	ND	Decapod (larvae)	N	NO (PCR 18S with SS2/SAS1 primers <sup>15</sup> )	ND	ND	ND	ND	NS	NS	NS	NS	NS	Audemard <i>et al.</i> , 2002
Order: Decapoda	ND	No common name	N	PCR targeting IGS and sequencing	ND	ND	ND	ND	NS	NS	NS	NS	NS	Carrasco <i>et al.</i> , 2007b
Class: Ostracoda	ND	No common name	N	NO (PCR 18S with SS2/SAS1 primers <sup>15</sup> )	ND	ND	ND	ND	NS	NS	NS	NS	NS	Audemard <i>et al.</i> , 2002
Annelida														
Spionidae	Streblospio shrubsolii	No common name	N	NO (PCR 18S with SS2/SAS1 primers <sup>15</sup> )	ND	ND	ND	ND	NS	NS	NS	NS	NS	Audemard et al., 2002
Class: Polychaeta	ND	No common name	N	PCR IGS	ND	ND	ND	NO	NS	NS	NS	NS	NS	Arzul et al., 2014
Tunicata														
Molgulidae	Molgula manhanttensis	Common sea grape	N	NO (PCR 18S with SS2/SAS1 primers <sup>15</sup> )	ND	ND	ND	ND	NS	NS	NS	NS	NS	Audemard <i>et al.</i> , 2002
Fritillariidae	Appendicularia sp.	No common name	N	PCR IGS	ND	ND	ND	NO	NS	NS	NS	NS	NS	Arzul et al., 2014
Chaetognatha														
Sagittidae	Sagitta sp.	No common name	N	PCR IGS and sequencing	ND	ND	ND	NO	NS	NS	NS	NS	NS	Arzul <i>et al.</i> , 2014
Cnidarians														
Sagartiidae	Cereus pedunculatus	No common name	N	NO (PCR 18S with SS2/SAS1 primers <sup>15</sup> )	ND	ND	ND	ND	NS	NS	NS	NS	NS	Audemard <i>et al.</i> , 2002
Nemertea														
Lineidae	Lineus viridis	No common name	N	NO (PCR 18S with SS2/SAS1 primers <sup>15</sup> )	ND	ND	ND	ND	NS	NS	NS	NS	NS	Audemard <i>et al.</i> , 2002
Pisces														
Gobiidae	Pomatoschistus microps (juveniles)	No common name	N	NO (PCR 18S with SS2/SAS1 primers <sup>15</sup> )	ND	ND	ND	ND	NS	NS	NS	NS	NS	Audemard <i>et al.</i> , 2002

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- <sup>6</sup> Morphology from histology was later characterised by linked molecular information from Le Roux et al. (2001).
- Mortality and hemocytic infiltration was reported. However, it cannot be concluded that the causative pathogenic agent was M. refringens as there was a co-infection with B. exitiosa.
- <sup>8</sup> In Ria de Arousa there are three species of *Marteilia* identified (*M. refringens; M. cochillia; M. octospora*). Without molecular information, it is difficult to conclude which *Marteilia* species is present in the animals sampled.
- <sup>9</sup> Animals sampled from a mortality event. However, it cannot be concluded that the causative pathogenic agent was *M. refringens*.
- 10 Experimental transmission assay (from copepod to mussels) was unsuccessful but it cannot be concluded that the parasite was non-viable.
- 11 Mortality was reported. However, it cannot be concluded that the causative pathogenic agent was M. refringens as there was a co-infection with B. ostreae.
- 12 Mortality was reported. However, there was insufficient information to be confident that it was associated with M. refringens.
- <sup>13</sup> No histology was completed and samples were from outside mortality event.
- <sup>14</sup> Phylogenetic analysis of sequences from the Genbank allowed conclusions on the type.
- <sup>15</sup> The 18S SS2/SAS1 primers are not specific enough to confirm *Marteilia refringens*.
- <sup>16</sup> PCR positive but the *ad hoc* Group concluded that contamination could not be ruled out.
- <sup>17</sup> The mortality that was reported was attributed to a haplosporidium.
- <sup>18</sup> Subsequent molecular testing from Le Roux et al. (2001) was not used because it did not include any information from this species.

#### **Assessment Table Key**

N: Natural infection

E: Experimental (non-invasive)

YES: Demonstrates criterion is met

NO: Criterion is not met

I: Inconclusive

ND: Not determined

NS: Not scored

#### 5. Naming convention for susceptible species

The scientific names of the host species are in accordance with the World Register of Marine Species (WoRMS) <a href="https://www.marinespecies.org/index.php">https://www.marinespecies.org/index.php</a>.

The common names of mollusc species are in accordance with FAOTERM (<a href="http://www.fao.org/faoterm/collection/faoterm/en/">http://www.fao.org/faoterm/collection/faoterm/en/</a>). Where the common mollusc name was not found in FAOTERM, the naming was done in accordance with <a href="https://www.sealifebase.ca">https://www.sealifebase.ca</a>.

#### 6. Comments on the ad hoc Group's rationale and decision-making

'Inconclusive' was used to distinguish situations where more information was provided than would have been assessed as 'Non-determined' but the *ad hoc* Group could not conclude that the criterion was met. Each time inconclusive was used within the assessment table, the *ad hoc* Group provided additional information in a footnote. The *ad hoc* Group treated 'Inconclusive' as 'Non-Determined' when making their final assessment.'

Where possible the *ad hoc* Group included information regarding the types but for a variety of reasons the *ad hoc* Group was rarely able to assess susceptibility of species at the type level.

At least three studies (Le Roux *et al.*, 2001; Novoa *et al.*, 2005; Lopez-Flores *et al.*, 2004) provided evidence for the co-occurrence of both types in several locations and within the same individual. Under those conditions, it is impossible to link molecular information regarding the genetic type with morphological and pathological information. These studies used a cloning approach to demonstrate the presence of both genetic types. When cloning is not used, e.g. when direct sequencing is employed, techniques can exclude the detection of one genetic type or the other. The majority of studies did not provide discrimination of the genetic types. The *ad hoc* Group did try to use later studies of the regionally occurring genetic types. However, when combining molecular and morphological and pathological information between studies, the survey designs, even when using cloning, were often not sufficiently representative (limited sample size and extent) to infer consistency of genetic types through time.

#### 6.1. General comments

The *ad hoc* Group agreed to focus on studies published from 2000 onwards, when molecular testing was available. Papers published in earlier years were referred to when necessary to increase confidence of an assessment or when no recent paper was available for the assessment of a specific host species. When necessary to corroborate pathogen identification, the *ad hoc* Group:

- (1) contacted authors of the studies to further describe pathogen identification methods, or
- (2) utilized molecular information from parallel or subsequent studies on the same source population.

The *ad hoc* Group agreed that while the ideal situation was two papers with a score of '1', a single study scoring '1' with corroborative evidence was also enough to conclude susceptibility of a species in the absence of conflicting evidence. Where sampling strategy was distributed across seasons or locations, and/or where a single paper provided all evidence (molecular with corresponding evidence from histology within the same animals), the *ad hoc* Group considered that one strong paper was sufficient to conclude susceptibility of a species. Consequently, additional studies were still reviewed to check for any supporting or conflicting evidence. When additional papers were identified but the *ad hoc* Group did not feel that they were necessary to assess because the species had already been determined as susceptible by other studies, these studies were included in the list of references.

#### 6.2. Species-specific comments

- Ostrea chilensis: only one study was available for assessment. The evidence provided was assessed by the ad hoc Group as having met the criteria for susceptibility and was scored as a '1'. However, the ad hoc Group was unable to find any additional studies or corroborative evidence within the Grizel et al., 1993 study. As a result, the ad hoc Group assessed Ostrea chilensis as an overall score of '2' and proposed it to be included in Section 2.2.2. of Chapter 2.4.4., Infection with Marteilia refringens, of the Aquatic Manual.
- Ostrea puelchana: the ad hoc Group were unable to score Ostrea puelchana, despite it being currently listed as susceptible in Article 11.4.2. While it is likely that the parasite identified in the Pascual et al., 1991 study is Marteilia refringens, due to the location of the study (different from the location for which subsequent molecular data are available in Le Roux et al., 2001), the evidence presented in the paper was not sufficient to conclude susceptibility.

- Ostrea stentina: in light of new scientific evidence and personal communications, the ad hoc Group recognises that Ostrea stentina and Ostrea equestris are considered distinct species. The ad hoc Group also noted that the two species had a different geographic distribution. Ostrea equestris is distributed in the Americas (North and South) and the western Pacific (New Zealand), while Ostrea stentina is distributed in the eastern Atlantic (Tunisia, Spain). For the purposes of assessment for susceptibility to infection with Marteilia refringens, all papers reviewed were located within Tunisia and Spain. Therefore, the ad hoc Group concluded that the species was in fact Ostrea stentina.
- Chamelea gallina: only one study was available for assessment. The evidence provided was assessed by the ad hoc Group as having met the criteria for susceptibility and was scored as a '1'. The ad hoc Group considered that the diagnostic testing outlined in Lopez-Flores et al. (2008b) which included molecular testing and histological evidence, was sufficient to assess it as a susceptible species.
- Solen marginatus: only two studies were available for assessment. The evidence provided was assessed by the ad hoc Group as having met the criteria for susceptibility and one of the studies was scored as a '1'. The ad hoc Group considered that the diagnostic evaluation outlined in Lopez-Flores et al. 2008a study which included both molecular pathogen identification and histological review of the same study population, was sufficient to assess it as a susceptible species.
- Xenostrobus securis: only one study was available for assessment. The evidence provided was assessed by the ad hoc Group as having met the criteria for susceptibility and was scored as a '1'. The ad hoc Group considered that the sampling strategy outlined in Pascual et al., 2010 study included multiple years of testing and the molecular testing and histological evidence was sufficient to assess it as a susceptible species.
- Magallana gigas also known as Crassostrea gigas:
  - To date, there have been no reports of the mature (tertiary) stages of *Marteilia refringens* in *Crassostrea gigas*. If this changes, this assessment would require re-evaluation.
  - According to WoRMS, the accepted name for Crassostrea gigas should be Magallana gigas. Previously the ad hoc Group had maintained the name as Crassostrea gigas based on the arguments provided by Bayne et al. (2017) and considered that the report by Salvi & Mariottini (2017) was not sufficiently robust to support the proposed taxonomic change. However, the ad hoc Group considered new data and peer reviewed publications (Salvi & Mariottini, 2020; Salvi et al., 2022; Sigwart et al., 2021) on the new name of Magallana gigas. Currently Magallana gigas is the accepted name in WORMS and Crassostrea gigas is considered an alternate representation in order to reflect the contrasting views of Byane et al. (2017). To ensure consistency with the approach of ensuring scientific names are in accordance with WORMS while recognising that Crassostrea gigas will be widely used, the ad hoc Group has agreed to identify Pacific cupped oyster as 'Magallana gigas also known as Crassostrea gigas' within the assessment table. The ad hoc Group recommended to the Commission that it be included as such in the Aquatic Code and the Aquatic Manual.

#### Mytilus edulis:

- Several papers reviewed by the ad hoc Group for the assessment of Mytilus edulis concerned mussels from La Trinité River. All these studies were scored as an outcome of 1. However, none of the studies reviewed by the ad hoc Group included the characterisation of the mussel species sampled. The ad hoc Group reviewed the geographic distribution of Mytilus galloprovincialis and Mytilus edulis and considered that this region has cohabitation of both species and hybrids. Bierne et al. (2003) showed that hybrids were present in La Trinité River. Therefore, the ad hoc Group could not be confident that the species sampled were Mytilus edulis and considered them to be mixed populations of M. edulis, M. galloprovincialis and their hybrids. Consequently these papers were not included in the final assessement of Mytilus edulis.
- Based on Bøgwald et al. (2022), the ad hoc Group was able to assess Mytilus edulis with a score 1. The evidence provided was assessed by the ad hoc Group as having met the criteria for susceptibility and was scored as a '1'. The ad hoc Group considered that the sampling strategy outlined in this study included multiple years of testing, the molecular testing and histological evidence, and was therefore sufficient to assess it as a susceptible species. In addition, the sampling was completed in an area where only Mytilus edulis is found. Michalek et al. (2016) provided general information about the distribution of mussel species in Europe.
- Mytilus galloprovincialis: based on Michalek et al. (2016), the studies reviewed did not raise questions about the species identity of M. galloprovincialis.

Ruditapes decussatus: although there was molecular detection of Marteilia refringens in conjunction with ISH results, the ad hoc Group considered that the intrepretation of the pictures provided within Boyer et al. (2013) supported that the clams were not infected with viable parasites. The evidence provided was assessed by the ad hoc Group as having met the criteria for a score of 3.

#### Zooplankton:

- Where the authors of studies reviewed did not provide the species name, the ad hoc Group stayed
  at a higher classification level: at the Order or Class level (for example, Order Harpacticoida for
  Harpacticoid in Carrasco et al., 2007a) or at the genus level (for example, Evadne sp. in Arzul et
  al., 2014).
- If only one sample was positive by PCR then the *ad hoc* Group considered it not to be a score "3" (PCR positive) but instead put it as a "NS" considering that contamination could not be ruled out. In order to assess a species as having met the criteria for a score "3", multiple positives could have come from a single or separate studies (for example, *Euterpina acutifrons*).
- Only *Paracartia grani* met the criteria to be scored as a "1" and this is based on the molecular information and the ISH results from multiple studies.
- Paracartia latisetosa also had molecular and ISH results but was assessed as a "2" because only
  two individuals tested positive from a single sampling event. Paracartia latisetosa should be
  reassessed if more information becomes available in the future.
- Oithona sp. from France and Spain (and two geographic locations within Spain) could not be assumed to be conspecific. The ad hoc Group assessed the studies individually and proposed them for inclusion in Section 2.2.2. of Chapter 2.4.4., Infection with Marteilia refringens, of the Aquatic Manual as unidentified copepod species of the genus Oithona.
- There are many species where there is no molecular information on the pathogen identification of M. refringens and therefore it was not possible to score these species. They were included within the table as NS.

#### 7. Article 1.5.9. Listing of Susceptible species at a taxonomic ranking of Genus or Higher

The *ad hoc* Group considered Article 1.5.9., Listing of susceptible species at a taxonomic ranking of Genus or higher in the *Aquatic Code*, and determined that it was not applicable for the susceptible host species for *M. refringens* identified at this time.

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.../Annexes

#### Annex I. List of Participants

#### MEETING OF THE WOAH AD HOC GROUP ON SUSCEPTIBILITY OF MOLLUSC SPECIES TO **INFECTION WITH OIE LISTED DISEASES**

#### November-December 2021 and May-June 2022

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#### Annex II: Terms of Reference

# WOAH *AD HOC* GROUP ON SUSCEPTIBILITY OF MOLLUSC SPECIES TO INFECTION WITH OIE LISTED DISEASES

#### November-December 2021 and May-June 2022

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## **Background**

Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogenic agent, was introduced in the 2014 edition of the *Aquatic Code*. The purpose of this chapter is to provide criteria for determining which host species are listed as susceptible in Article X.X.2. of each disease-specific chapter in the *Aquatic Code*. The criteria are to be applied progressively to each disease-specific chapter in the *Aquatic Code*.

These assessments will be undertaken by *ad hoc* Groups and the assessments will be provided to Members for comment prior to any change in the list of susceptible species in Article X.X.2. of the disease-specific chapters in the *Aquatic Code*.

For species where there is some evidence of susceptibility but insufficient evidence to demonstrate susceptibility through the approach described in Article 1.5.3., information will be included in the relevant disease-specific chapter in the *Aquatic Manual*.

#### **Purpose**

The WOAH *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases will undertake assessments for infection with *Marteilia refringens* in molluscs.

#### **Terms of Reference**

- 1) Consider evidence required to satisfy the criteria in Chapter 1.5.
- 2) Review relevant literature documenting susceptibility of species for infection with Marteilia refringens.
- 3) Propose susceptible species for infection with *Marteilia refringens* based on Article 1.5.7.
- 4) Propose a list of susceptible species of molluscs for infection with Marteilia refringens based on Article 1.5.8.

#### Expected outputs of the ad hoc Group

- 1) Develop a list of susceptible species for inclusion in Article 11.4.2. in the *Aquatic Code*.
- 2) Develop a list of species with incomplete evidence for susceptibility for inclusion in Section 2.2.2. of the *Aquatic Manual*.
- 3) Draft a report for consideration by the Aquatic Animals Commission at its February 2022 meeting.

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