



# Alien parasitic copepods in mussels and oysters of the Wadden Sea

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1   **Alien parasitic copepods in mussels and**  
2   **oysters of the Wadden Sea**

3

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21    **Abstract**

22    Molluscan intestinal parasites of the genus *Mytilicola*, specifically *M. intestinalis*,  
23    were initially introduced into bivalves in the North Sea in the 1930s. It was  
24    presumably introduced from the Mediterranean with ship-fouling mussels, then  
25    attained epidemic proportions in *Mytilus edulis* in the 1950s, and is now widely  
26    established in the North Sea region. *Mytilicola orientalis* was co-introduced with  
27    Pacific oysters to France in the 1970s and in the southern North Sea in the early  
28    1990s. Its main host *Crassostrea gigas* has massively invaded the Wadden Sea  
29    with a concomitant decline in mussels. To explore whether introduced mytilicolid  
30    parasites could play a role in the shifting dominance from native mussels to  
31    invasive oysters, we analyzed 390 mussels and 174 oysters collected around the  
32    island of Sylt in the northern Wadden Sea. We show that *M. intestinalis* has a  
33    prevalence >90% and a mean intensity of 4 adult copepods in individual mussels  
34    with >50 mm shell length at all sheltered sites. By contrast, none were found in  
35    the oysters. However, at one site we found *M. orientalis* in *C. gigas* with a  
36    prevalence of 10% and an intensity of 2 per host individual (August 2008). This  
37    constitutes the most northern record in Europe for this Pacific parasite until now.  
38    Alignments of partial sequences of the mitochondrial cytochrome oxidase I (COI)  
39    gene and the nuclear internal transcribed spacers (ITS) and 18S rDNA-sequences,  
40    each show a distinct difference between the two species which confirms our  
41    morphological identification. We suggest that the high parasite load in mussels  
42    compared to oysters may benefit the continued expansion of *C. gigas* in the  
43    Wadden Sea.

44  
45    **Keywords:** *Introduced species, Mussels, Mytilicola, Oysters, Parasites, Wadden*  
46    *Sea*

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## 55 **Introduction**

56 Invasions of alien species have become a major process of change in marine  
57 coastal ecosystems (Ruiz et al. 1997; Reise et al. 2006; Rilov and Crooks 2009).  
58 Parasites may have two different effects on this global process. On the one hand,  
59 introduced species often escape from their native parasites during transport and  
60 when founder populations are rather small (Torchin and Lafferty 2009). In  
61 addition, parasites with complex life cycles seldom encounter all necessary hosts  
62 in a recipient ecosystem and thus are rarely co-introduced (Torchin et al. 2003),  
63 and native parasites tend to infest alien hosts only at low numbers (Krakau et al.  
64 2006). This parasite release is assumed to generally facilitate invasive hosts.  
65 On the other hand, with transfers of organisms for aquaculture and to some extent  
66 also with ships or the aquaria trade, diseases and parasites have been introduced  
67 with occasionally disastrous effects when they infest naïve (previously  
68 unexposed) host species in a recipient coastal region (Cook et al. 2008; Minchin et  
69 al. 2009; Pillay 2004). Particularly, oyster stocks have been strongly affected by  
70 introduced disease agents (Andrews 1980; Wolff and Reise 2002). Many of these  
71 parasites mature and reproduce on or in a single host, and spores or larvae  
72 disperse to the next host individual (direct life cycle). This makes their invasion  
73 more likely compared to parasites with complex life cycles which depend on the  
74 presence of all hosts to become established. Parasitic copepods of the genus  
75 *Mytilicola* exemplify the type of invasive parasites with direct life cycles. They  
76 inhabit the guts of molluscs and disperse via larval stages to infect other host  
77 individuals (Lauckner 1983, Gotto 2004). In European seas, *M. intestinalis* has  
78 been described from the mussel *Mytilus galloprovincialis* in the Adriatic Sea by  
79 Steuer (1902). On the Atlantic coast, it appeared in *M. edulis* in England and in  
80 the German Bight in the 1930s (Caspers 1939; Korrynga 1968) and is believed to  
81 have been imported with mussels fouling ship hulls (Korrynga 1968).

82 However, for British waters Eno et al. (1997) have not included *M.*  
83 *intestinalis* as an introduced species, based on a pers. comm. by M. Gee “that its  
84 biology suggests that it is a boreal species” (p.12). This remark seems to be  
85 derived from results in Gee and Davey (1986) showing that development can be  
86 completed at temperatures below 18°C albeit at slow rates. We follow Korrynga  
87 (1968) who assumes it as very unlikely that this highly conspicuous parasite has

88 been overlooked in North Sea mussels prior to the 1930s, and thus regard *M.*  
89 *intestinalis* as an introduced rather than being a cryptogenic species.

90 From 1949 to 1951 an epidemic was observed at the North Sea coast, and *M.*  
91 *intestinalis* was blamed for massive mortality in *M. edulis* with a severe impact on  
92 the mussel fishery (Korringa 1968). Although this parasite evidently can harm its  
93 host (e.g. Meyer and Mann 1950; Williams 1969; Mann 1979; Theisen 1987),  
94 doubts have been raised as to whether *M. intestinalis* has been the causal agent for  
95 the observed mortality in the mussel epidemic or as to whether it merely had  
96 spread coincidentally at that time (Detlefsen 1975; Lauckner 1983).

97 A second *Mytilicola* species, *M. orientalis* has been described by Mori (1935)  
98 from the Pacific oyster *Crassostrea gigas* and mussels in the Inland Sea of Japan,  
99 and this species was first introduced with its host *C. gigas* to the Pacific coast of  
100 North America in the 1930s and to France in the 1970s (Katkansky et al. 1967;  
101 His 1977; His et al. 1978; Lauckner 1983; Grizel 1985). On the European Atlantic  
102 coast it has spread further to the Netherlands and Ireland (Stock 1993; Holmes  
103 and Minchin 1995), and has also been introduced with *C. gigas* into the  
104 Mediterranean Sea (Clantzig 1989). Although it was initially feared that *M.*  
105 *orientalis* could initiate an epidemic similar to the one assumed with *M.*  
106 *intestinalis* in 1949-51, this has not yet been observed, and harm to the host *C.*  
107 *gigas* seems to be small (Deslous-Paoli and Heral 1988; DeGrave et al. 1995;  
108 Steele and Mulcahy 2001).

109 In the northern Wadden Sea, the typical hosts for these two parasites co-  
110 occur. While the mussels *M. edulis* are native, the oysters *C. gigas* have recently  
111 been introduced. Regular imports of young Pacific oyster *C. gigas* for sea  
112 ranching at Sylt island have occurred since 1986, and the oysters subsequently  
113 invaded mussel beds in that area (Reise 1998; Diederich et al. 2005). The question  
114 arose as to whether *C. gigas* could displace the native mussels (Diederich 2005,  
115 2006; Nehls et al. 2006). Direct competition between mussels and oysters for  
116 space and food has been suggested (Troost 2009), but it also seems likely that  
117 indirect competition mediated by differential parasite loads among the two host  
118 species (apparent competition) could be another contributing factor (Krakau et al.  
119 2006).

120 The aim of our study was to investigate mussels and oysters for the presence  
121 of the two parasitic copepod species and to quantify the respective infection levels

122 in their hosts. To test the selectivity of the infestation, we experimentally  
123 investigated the host specificity of *M. intestinalis* in mussels and oysters. Due to  
124 ambiguous features of species identification mentioned in the literature (Stock  
125 1993, Gotto 2004) we sequenced parts of the nuclear rDNA and one  
126 mitochondrial gene to genetically verify species determination based initially on  
127 morphological features only.

## 129 **Material and methods**

### 130 **Sampling area**

131 The 390 individuals of *Mytilus edulis* and 174 of *Crassostrea gigas* examined in  
132 this study were sampled at 6 sites around the island of Sylt in 2007 and 2008 (Fig.  
133 1, Tables 1 and 2). The intertidal sampling sites in the north and in the south are  
134 disconnected by a dam from the island to the mainland since 1927. Sampling site  
135 3 at the exposed beach refers to mussels attached to groins. All other mussels and  
136 the oysters collected were living on sediment in the low tidal zone and at one site  
137 in the shallow subtidal zone. The subtidal mussels collected in August 2007 were  
138 dredged at a depth of 1-2 m, while the ones collected in May 2008 were collected  
139 by hand at a depth of about 0.5 m during extreme low tide. Regarding the two  
140 sites sampled for both mussels and oysters, the oyster bank was approximately  
141 500 m from the mussel bank at site 2, whereas mussels and oysters were co-  
142 occurred in a mixed assemblage at site 6. For every host individual, the infestation  
143 of *Mytilicola* spp. was recorded to determine the mean prevalence (% of host  
144 individuals infected) and the mean intensity (mean number of parasite individuals  
145 per infected host individuals) of the two copepods in each host population.

146       Mussels were divided into three shell length categories: small (<30 mm),  
147 medium (30 mm to 50 mm) and large (>50 mm; cp. Meyer and Mann 1950).  
148 These categories have previously been shown to be positively correlated with  
149 prevalence and intensity of *M. intestinalis* (e.g. Detlefsen 1975). Due to the fact  
150 that the last recruitment event in *C. gigas* had occurred in summer 2006, small  
151 oysters were not available and random samples were taken.

152 After mussels and oysters were opened, the visceral mass was taken out and  
153 squeezed between two glass plates. Only adult stages of *Mytilicola* spp. were  
154 counted. These are readily detected due to their size and intensive red colour.

155 To detect statistically significant differences in infection levels, prevalence  
156 and intensity data were compared using Chi-Square-Test and analysis of variance  
157 (ANOVA), respectively.

158

#### 159 Infection experiment

160 In order to test host specificity of *M. intestinalis* with regard to *M. edulis* and *C.*  
161 *gigas* a pilot study was conducted from the beginning of September to the  
162 beginning of November 2008. We infected previously uninfected individuals  
163 (medium-size mussels from site 3 and oysters from site 2, recipient mussels and  
164 oysters) by keeping them together in treatments with highly infected large blue  
165 mussels (from site 2, donor mussels) for two months. Recipient mussels were  
166 marked and could readily be distinguished by their size. The average rate of  
167 infection of recipient mussels and oysters and donor mussels was determined  
168 before the experiment commenced with samples of n=30 for each host species  
169 (see Tab. 1 and 2, samples from 2008). Epibionts of mussels were removed prior  
170 to the experiment.

171 Twelve aquaria (20 l) were used, each containing 10 donor mussels. Recipient  
172 mussels and oysters were exposed to infected mussels separately to prevent  
173 mutual interference. Therefore, 6 treatments were used for the infection of  
174 mussels and 6 for the infection of oysters. Of the recipient mussels and oysters, 6  
175 individuals were added to each treatment. The bivalves were kept in oxygenated  
176 sea water filtered with a gravel filter. Every day, 3 l of each tank were renewed.  
177 Once every week, the whole tank was filled with fresh filtrated sea water.  
178 Previously uninfected mussels and oysters (n=20 each) were kept as a control in  
179 unfiltered seawater. Water temperature remained at 15°C during the entire  
180 experiment. After two months, all individuals were simultaneously taken out of  
181 the water and stored at 4°C until dissection within 2 weeks. Cooled storage is  
182 assumed to have no effect on the intensity of the infestation (Dethlefsen 1972).

183

## 184 Genetic species differentiation

185 For the genetic differentiation of the species, the DNA of 5 specimens of  
186 *Mytilicola intestinalis* and 2 of *M. orientalis* was extracted using the Qiagen  
187 DNeasy Blood and Tissue Kit. From this extracted DNA, the sequences of the  
188 18S rDNA, the internal transcribed spacers (ITS) of the rDNA and sequences of  
189 the 5' part of the mitochondrial cytochrome c oxidase I (COI) gene were  
190 determined after amplification using Polymerase chain reaction (PCR). Due to its  
191 length, the 18S sequence was divided into 3 overlapping parts which were  
192 separately amplified using 3 sets of forward and reverse primers. All primers used  
193 are specified in Table 3. The annealing temperature was set to 57°C for the 18S  
194 primers and to 50°C for the COI and ITS primers. The PCR products were  
195 purified using the Qiagen QIAquick PCR Purification Kit and sent to Eurofins  
196 MWG Operon for sequencing. The three parts of the 18S sequence were manually  
197 rejoined to one sequence of 1728 base pairs based on the overlapping regions. The  
198 sequences are available in GeneBank under the accession numbers HM775187 –  
199 HM775206.

200 The 18S rDNA, the COI and the ITS sequences of *M. intestinalis* and *M.*  
201 *orientalis* were inserted into an alignment using ClustalX (Higgins and Sharp  
202 1988). Based on this alignment, the percentage of differing bases was calculated.  
203 All three genomic regions were determined in both *M. orientalis* individuals  
204 analysed. Regarding *M. intestinalis*, COI and ITS sequences were obtained from  
205 all five individuals while for the 18S sequencing, only two individuals were used.  
206

## 207 Results

### 208 Infection levels in mussels and oysters

209 Almost all mussel populations were infected with *M. intestinalis* and all mussels  
210 were free of *M. orientalis* (Tables 1 and 2). Prevalence and intensity were  
211 generally high in large mussels (>90% and approx. 4 parasites/host), only medium  
212 mussels from the exposed shore of the island (site 3) had very low infection  
213 values (Table 1). In contrast, oysters were free of *M. intestinalis* and *M. orientalis*  
214 was found only at a single sampling site (Fig. 1, site 6; 54.7917°N, 8.3056°E). At



215 this site, infection levels of *M. intestinalis* in mussels were significantly higher  
216 than of *M. orientalis* in oysters (Fig. 2; prevalence:  $\chi^2=45.3$ ; intensity:  $F_{59}=44.3$ ,  
217  $p<0.001$ ).

218

#### 219 Experimental infections

220 This pilot experiment yielded no infection of oysters but a noticeable infection of  
221 previously uninfected mussels (recipient mussels). Approximately half of the  
222 recipient mussels in each aquarium were infected (mean prevalence  $45.6\pm7.2\%$ ,  
223  $n=35$ ) with one or two *M. intestinalis* in each mussel (mean intensity  $1.3\pm0.3$ ).  
224 After the experiment, the donor mussels used for the infection had a prevalence of  
225 93.3% and an intensity of  $5.3\pm3.5$ . (The control mussels, previously uninfected  
226 but kept in unfiltered seawater, unexpectedly showed similar infection rates  
227 (prevalence 30.0% and intensity  $1.7\pm0.9$ ). Again, oysters remained uninfected.)

228

#### 229 Genetic species differentiation

230 A comparison of the *M. intestinalis* and *M. orientalis* sequences obtained showed  
231 notable differences regarding intra- and interspecific variation. Intraspecifically,  
232 no sequence variation existed between the analysed individuals of *M. intestinalis*  
233 (based on five individuals for the COI and ITS sequences and two individuals for  
234 the 18S region). For the two *M. orientalis* individuals analysed, intraspecific  
235 variations were found in all three sequences, although they are well under 1%  
236 (Table 4).

237 The interspecific differences are much higher. Regarding the conserved 18S  
238 sequence, they exceed 1%. For COI and ITS, both sequence markers with higher  
239 mutation rates, differences above 10% were determined.

240 During our investigations, we noticed that morphological identification  
241 features mentioned by Stock (1993) and Gotto (2004) are problematic. The caudal  
242 rami are said to be strongly divergent in *M. intestinalis* and almost parallel in *M.*  
243 *orientalis*. According to our observations, this feature is not reliable because  
244 *Mytilicola* can actively pull on the caudal rami causing them to lie parallel or to  
245 spread them out until they lie at a 180 degree angle to each other. We found the  
246 dorsal appendages to be the most suitable feature for identification: they are short

247 and rounded in *M. intestinalis* and distinctly pointed and elongated in *M.*  
248 *orientalis* (Fig. 3).

## 250 Discussion

251 In the northern Wadden Sea we found a heavy infestation of native mussels  
252 *Mytilus edulis* by the intestinal mytilicolid copepod *Mytilicola intestinalis*,  
253 indicating that this introduced parasite has become well established since the first  
254 record in 1970 (Dethlefsen 1972), while still absent in 1950 and 1965 (Meyer and  
255 Mann 1950; Theisen 1966). Prevalence and intensity in 2003-04 (Thieltges et al.  
256 2006, 2008) and in 2007-08 (this study) have been at a similarly high level. In the  
257 invasive Pacific oyster *Crassostrea gigas* we found no *M. intestinalis*. However,  
258 at a single site in the south of the island of Sylt, far away from the local oyster  
259 culture lot in the north of the island, we found for the first time oysters infected  
260 with *M. orientalis*. The nearest previously reported occurrence is the Rhine Delta  
261 in The Netherlands (Stock 1993), approximately 570 km along the North Sea  
262 shore in south-western direction. We speculate that this parasite may have just  
263 arrived at this northern most location for this Pacific species in Europe, although  
264 this site has not been screened previously. Thus, the sweeping invasion of *C.*  
265 *gigas* in the northern Wadden Sea presumably took place in the absence of this  
266 parasite burden.

267 The presence of both parasitic copepods was confirmed by the molecular  
268 analyses. Avise (2000) suggests < 1% for intraspecific and > 1% for interspecific  
269 COI variation. We found differences of > 1% even in the conserved 18S sequence  
270 while COI sequences differed between *M. intestinalis* and *M. orientalis* by around  
271 16%. Thus, even when the limit of 1% for species differentiation based on COI  
272 variation may be low, our results reveal a clear genetic distinction between both  
273 species and point to a not very close phylogenetic relationship. This is supported  
274 by a comparison with a GeneBank entry of the 18S sequence of a third species of  
275 the Mytilicolidae, *Trochicola entericus* (Acc-No. AY627006). Differences  
276 between *M. intestinalis* and *T. entericus* (1.32%) were higher than between *M.*  
277 *intestinalis* and *M. orientalis* (max. 1.26%).

278 Lauckner (1983) reviewed the host spectra of *M. intestinalis* and *M.*  
279 *orientalis*, listing a number of bivalve species and even some gastropods. Mytilids  
280 may even occasionally harbour both species concurrently in southern France (His

1977). However, *M. intestinalis* has not been recorded from Pacific oysters, except in a study by Aguirre-Macedo and Kennedy (1999) who refer to this species occurring throughout the year in *C. gigas* collected at the Exe estuary in southern England. This is in contrast to our observations since we did not find *M. intestinalis* in the 174 oysters screened for parasites, although all oysters were collected at sites where *M. intestinalis* occurred abundantly in mussels, often found in aggregates with the oysters. By referring to the above mentioned problem in species identification, we suggest that the only mytilid copepod in the intestine of *C. gigas* is *M. orientalis*.

Our experimental infections support our field observations that oysters are unsuitable as a host for *M. intestinalis*, as we yielded a high prevalence in mussels whereas the oysters remained free of this parasite. (The unexpected infection of mussels kept separate from mussels with *M. intestinalis* was most likely due to the fact that the water supply system for the flow-through tanks of the experiment has its incurrent siphon not far away from a subtidal mussel bed which is heavily infected by *M. intestinalis*.) Nevertheless, this pilot experiment may indicate that Pacific oysters are not infected by *M. intestinalis*. Unfortunately, we could not get enough *C. gigas* with *M. orientalis* to conduct the reverse infection experiment.

That *M. orientalis* was encountered only at a single site in *C. gigas* near the southern tip of the island was unexpected because the only oyster farm in the region is near the northern tip of the island, and this company regularly imports young oysters from Irish sources where *M. orientalis* has already been reported to infest *C. gigas* (Holmes and Minchin 1995; Steele and Mulcahy 2001). The sites in the north and south of Sylt are separated by a dam connecting the island with the mainland (see Fig.1). Natural dispersal has to take a route all along the exposed shore of the island where *C. gigas* does not occur, and this amounts to a distance of at least 50 km, mostly against the residual currents. Thus, maybe it is more likely that *M. orientalis* has arrived in the northern Wadden Sea by spreading from the south within a *C. gigas* population which originates from cultures in the delta area of The Netherlands. Site 6 is located in an area with intensive mussel cultures with seed mussels taken from various sources. Pacific oysters could have been transferred together with mussels.

According to the literature, *M. intestinalis* has little or no effects on mussel growth and reproduction performance when intensity is low but negative effects

are evident at intensities of 3 and higher (Meyer and Mann 1950; Gee and Davey 1986; Theisen 1987). Likewise, *M. orientalis* does not harm its host *C. gigas* at low intensities (Steele and Mulcahy 2001), but has a negative effect at intensities of 3 and more (Odlaug 1946; Katkansky et al. 1967; Deslous-Paoli 1981). At the sampling site where both parasites occur, *M. intestinalis* has an intensity of about 4 in *M. edulis*, while that of *M. orientalis* was only about 2 in *C. gigas*. Thus, mussels are likely to suffer from its intestinal parasite while the oysters are probably not. This suggests that the invasive oysters may have a competitive advantage over the native mussels in the Northern Wadden Sea as a result of their unequal parasite burdens. However, differential loads with parasitic copepods are not the only competitive advantage of oysters. They may also be less susceptible to predators (crabs, shrimp, starfish), presumably due to their firm attachment, flat shape when small and with a sturdy shell when larger (own observation). And they also show low burdens of trematode parasites which regularly infect the native mussels (Krakau et al. 2006). The differential infestation of native mussels and invasive oysters with mytilicolid parasites may thus have contributed to the success of the oyster invasion. However, this difference may be attributed to the fact that *M. orientalis* has arrived very recently, six decades after the introduction of *M. intestinalis* in the North Sea region and nearly four decades after *M. intestinalis* was first found where we now have encountered *M. orientalis*. Future studies will have to show whether *M. orientalis* can attain the same level of infestation in *C. gigas* as *M. intestinalis* does in *M. edulis*.

337

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344

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 479



480   Legends

481

482   **Figure 1:** Sampling sites of mussels (squares) and oysters (asterisks) around the North Sea island  
483   of Sylt. 1 Ellenbogen, 2 List, 3 Kampen, 4 Leghörn, 5 Munkmarsch, 6 Puan Klent. Gray shaded  
484   areas indicate the intertidal zone

485

486   **Figure 2:** Prevalence and intensity of *Mytilicola intestinalis* in its host *Mytilus edulis* and of *M.*  
487   *orientalis* in its host *Crassostrea gigas* at sampling site 6 in August 2008 (see Fig. 1, Tab. 1 and  
488   2). n=30 for each. \*statistically significant difference

489

490   **Figure 3:** *Mytilicola intestinalis* (A, B) and *M. orientalis* (C, D) in dorsal (A, C) and ventral (B,  
491   D) view. Female with egg sacks (one missing in *M. orientalis*), and dwarf male in each figure.  
492   Loss of the intense red colour due to ethanol fixation. Note the dorsal protuberances (arrowheads)  
493   as a distinct differential character

494

495 **Table 1:** *Crassostrea gigas* investigated for intestinal copepods with locality (see Fig.1), date,  
 496 number, shell length (mean  $\pm$  standard deviation) prevalence (% of host individuals infected) and  
 497 intensity (mean  $\pm$  standard deviation). Only *Mytilicola orientalis* was found in *C. gigas*  
 498

Site	Date	Oysters n	Shell length(mm)	Prevalence (%)	Intensity $\pm$ SD
1	May 2008	30	95.3 $\pm$ 28.4	0.0	0.0
2	August 2007	19	80.8 $\pm$ 26.5	0.0	0.0
2	August 2008	55	91.0 $\pm$ 21.5	0.0	0.0
4	May 2008	10	128.1 $\pm$ 20.0	0.0	0.0
5	May 2008	30	107.0 $\pm$ 24.7	0.0	0.0
6	August 2008	30	106.2 $\pm$ 22.1	10.0	1.7 $\pm$ 0.6

499

500

501 **Table 2:** *Mytilus edulis* investigated for intestinal copepods with locality (see Fig.1), date, number,  
 502 shell length (mean  $\pm$  standard deviation) prevalence (% of host individuals infected) and intensity  
 503 (mean  $\pm$  standard deviation). Only *Mytilicola intestinalis* was found in *M. edulis* \*subtidal, all  
 504 others intertidal

505

506

Site	Date	Mussels n	Shell length (mm)	Category	Prevalence (%)	Intensity $\pm$ SD
2	August 2007	30	24,1 $\pm$ 3,9	small	33.33	1.60 $\pm$ 0.97
2	August 2007	30	38,1 $\pm$ 5,1	medium	63.33	4.05 $\pm$ 2.98
2	August 2007	30	57,6 $\pm$ 6,4	large	90.00	4.48 $\pm$ 3.32
2*	August 2007	30	72,7 $\pm$ 6,4	large	93.33	8.07 $\pm$ 4.75
2*	May 2008	30	70,3 $\pm$ 8,1	large	86.67	3.81 $\pm$ 2.95
2*	August 2008	120	68,0 $\pm$ 8,1	large	93.30	5.28 $\pm$ 3.53
3	August 2007	30	35,7 $\pm$ 3,1	medium	3.33	1.0 $\pm$ 0.18
3	May 2008	30	32,7 $\pm$ 1,6	medium	0.0	0.0 $\pm$ 0.0
6	August 2007	30	60,1 $\pm$ 4,2	large	93.33	4.18 $\pm$ 3.25
6	August 2008	30	66,5 $\pm$ 3,7	large	96.67	3.72 $\pm$ 2.76

507

508

509

510 **Table 3:** Name and sequence of primers used for PCR amplification and sequencing  
511

Primer name	Sequence 5' → 3'	Reference
COI f	GGTCAACAAATCATAAAGATATTGG	Folmer et al. 1994
COI r	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. 1994
ITS1 f	TCCGTAGGTGAACCTGCGG	White et al. 1990
ITS4 r	TCCTCCGCTTATTGATATGC	White et al. 1990
18S f	TACCTGGTTGATCCTGCCAG	Huys et al. 2006
18S 554 f	AAGTCTGGTGCCAGCAGCCGC	Huys et al. 2006
18S 1150 f (p2)	ATTGACGGAAGGGCACCACCAG	Huys et al. 2006
18S 614 r	TCCACCTACGAGCTTTTAAACC	Huys et al. 2006
18S 1282 r	TCACTCCACCACTAAGAACGGC	Huys et al. 2006
18S r	TAATGATCCTTCCGCAGGTTCAC	Huys et al. 2006

512  
513 **Table 4:** Inter- and intraspecific sequence differences determined in *M. intestinalis* and *M.*  
514 *orientalis*. For interspecific values, minimal (min) and maximal (max) values are shown  
515

Sequence	Base pairs	interspecific (%,	<i>M. intestinalis</i>	<i>M. orientalis</i>
		min / max)	intraspecific (%)	intraspecific (%)
18S	1744	1.20 / 1.26	0.0	0.06
COI	628	16.08 / 16.40	0.0	0.64
ITS	515	14.17	0.0	0.19

516  
517

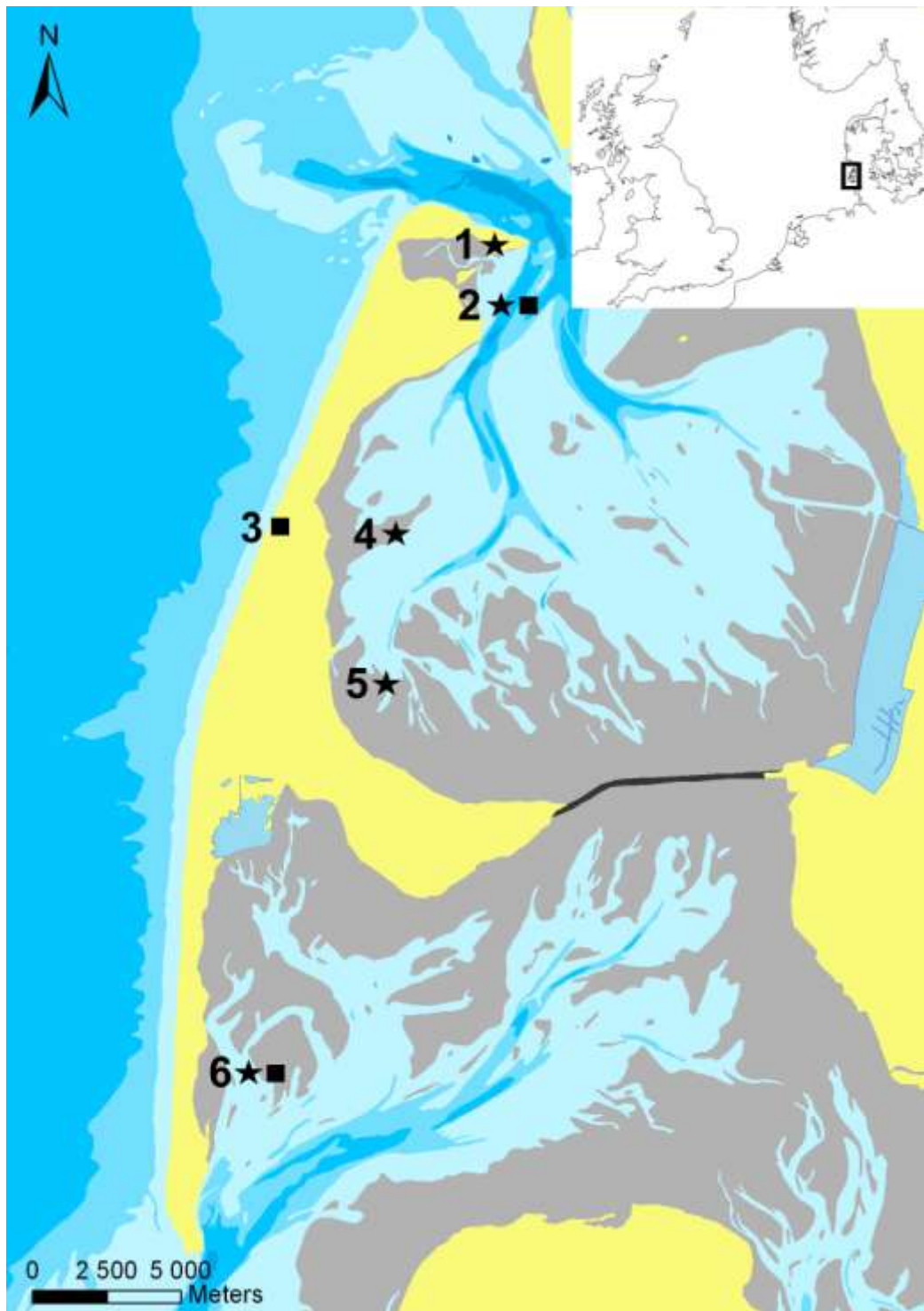
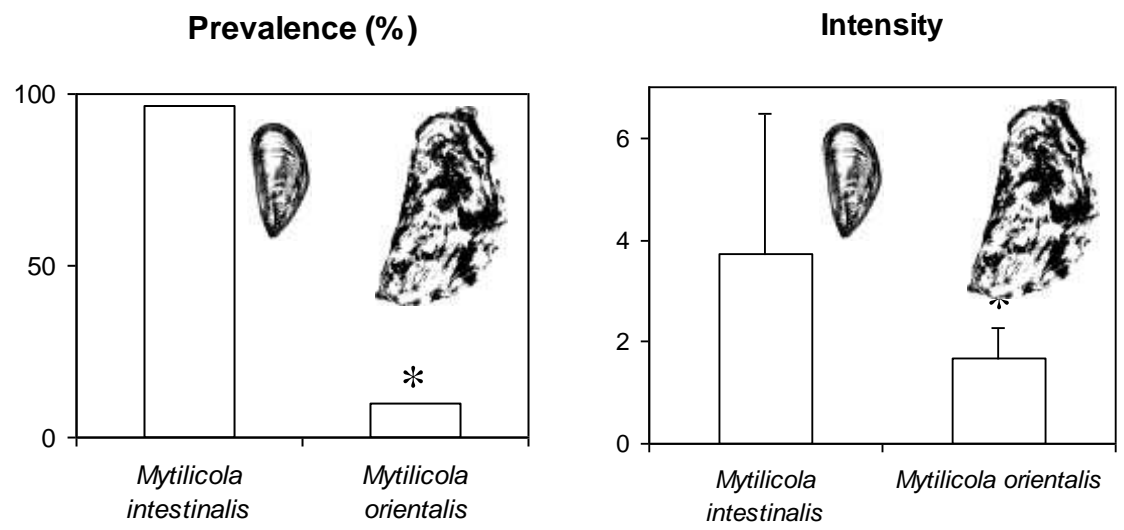
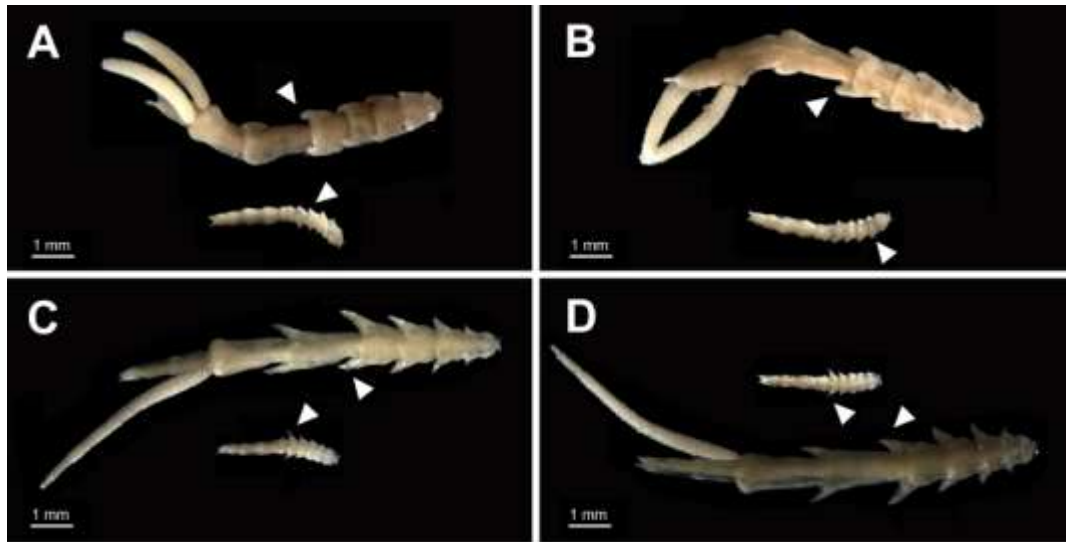


Figure 1



521  
522 Figure 2  
523



524

525 Figure 3