



Evaluating the genetic effects of the invasive *Ocenebra inornata* on the native oyster drill *Ocenebra erinacea*

Eric Pante, Pierre-Yves Pascal, Vanessa Becquet, Amélia Viricel, Benoit Simon-Bouhet, Pascale Garcia

► To cite this version:

Eric Pante, Pierre-Yves Pascal, Vanessa Becquet, Amélia Viricel, Benoit Simon-Bouhet, et al.. Evaluating the genetic effects of the invasive *Ocenebra inornata* on the native oyster drill *Ocenebra erinacea*. *Marine Ecology*, 2015, 36 (4), pp.1118-1128. 10.1111/maec.12208 . hal-01064464

HAL Id: hal-01064464

<https://hal.science/hal-01064464>

Submitted on 17 Sep 2014

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 **Evaluating the genetic effects of the invasive *Ocenebra inornata* on the native oyster drill**

2 ***Ocenebra erinacea***

3

4 Eric Pante¹, Pierre-Yves Pascal², Vanessa Becquet¹, Amélia Viricel¹, Benoit Simon-Bouhet¹,
5 and Pascale Garcia¹

6

7 ¹ Littoral, Environnement et Sociétés (LIENSs) UMR 7266 CNRS – Université de La
8 Rochelle, 2 rue Olympe de Gouges, 17000 La Rochelle, France.

9 ² Systématique-Adaptation-Evolution, Equipe Biologie de la Mangrove, Laboratoire de
10 Biologie Marine, UMR 7138 CNRS-MNHN-UPMS, Université des Antilles et de la Guyane,
11 BP 592 F-97159 Pointe-à-Pitre cedex, Guadeloupe, France

12

13 Keyword: *Ocenebra erinaceus*; *Ocenebrellus inornatus*; haplotype diversity; evolutionary
14 response; evolutionary ecology; invasive species; glacial refugium; cryptic species

15

Abstract

Studies focusing on the effect of invasive species on the genetic diversity of native marine invertebrates remain scant. Here we report diversity among French populations of the intertidal gastropod *Ocenebra erinacea* (Linnaeus, 1758) sampled in the presence and absence of the invasive *Ocenebra inornata* (Recluz, 1851). Between 1999 and 2004 a total of 352 individuals of *O. erinacea* were collected from 15 sites, five of which in the presence of the invasive, and genotyped at the mitochondrial locus *coxI*. No statistical difference was observed between polymorphism levels recorded within native populations exposed to the invasive, compared to populations sampled in the absence of *O. inornata*. No sign of native population decline was detected in response to the invader. While significant shifts in native *O. erinacea* population sizes were previously reported in the literature, genetic effects may take longer to accumulate, or may be undetectable without a larger panel of genetic markers. In contrast, large genetic distances and significant population differentiation were recorded between Atlantic and Mediterranean *O. erinacea* samples, suggesting that these populations have distinct evolutionary histories. Comparison of genetic divergence within the closely-related genus *Nucella* suggests that the Atlantic populations and the Mediterranean *O. erinacea* populations from Thau Lagoon may belong to different species or subspecies.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

35 **Introduction**

36

37 Natural movements of species ranges on the surface of the world (expansions,
38 regressions, displacements) play a considerable role in the evolution of species. Most of the
39 time, these phenomena are progressive and marked by the tempo of geological processes (e.g.
40 Hewitt 1996). However, during the last few decades a growing number of species has
41 undergone changes in their natural range due to both changes at a global scale (e.g. Parmesan
42 & Yohe 2003) and artificial transfers due to human activities (e.g. Carlton 1989; Seebens *et al.*
43 2013).

44 The number of biological invasions has strongly increased during the last decades (e.g.
45 Mack *et al.* 2000; Mooney & Cleland, 2001; Ruiz *et al.* 2000). In the marine environment,
46 these phenomena are mainly due to aquaculture and especially to shellfish farming, which
47 represents a major cause of introduction, intentional or not, of exogenous species (Elton 1958;
48 Carlton 1992).

49 Apart from potentially important economic consequences, the arrival of these
50 introduced species can also cause serious ecological impacts on local fauna. Introduced
51 species are likely to decrease the abundance of indigenous species, excluding them from part
52 of their distributional area or even causing their extinction by modifying invaded habitats,
53 hybridizing with native species, exchanging pathogens, preying on them, or competing with
54 them (e.g. Lockwood *et al.* 2007 and references therein). Moreover, when they exert strong
55 selective pressures, introduced species can also reduce the genetic diversity of native
56 populations (Kim *et al.* 2003; Wittmann *et al.* 2013).

57 Such impacts, although poorly known (e.g. Strauss *et al.* 2006), may have heavy
58 ecological consequences since adaptive potential depends on the genetic diversity of a

population. Reductions in genetic diversity are generally considered detrimental (e.g. Frankham 1995; Lande 1995; Strauss *et al.* 2006) and might contribute to extinction (Wittmann *et al.* 2013). Thus, various authors showed a link between the fitness of a species and its genetic diversity, particularly in mollusks (Mitton & Grant 1984; Garton & Haag 1991; Zouros 1993; Launey & Hedgecock 2001; Hedgecock *et al.* 2007). In addition, the reduced genetic variability of an indigenous population could promote the expansion process of other species that are phylogenetically close. However, very few studies address changes in genetic diversity of an indigenous species under the competitive pressure generated by a biological invader.

The oyster drills *Ocenebra erinacea* (Linnaeus, 1758) and *Ocenebra inornata* (Recluz, 1851) constitute a noteworthy model to study the genetic effects of indigenous-invasive interactions on indigenous populations. A native of the northwestern Pacific (Choe & Park 1997; Amano & Vermeij 1998), *O. inornata* (previously known as *Ocenebrellus inornatus*; see Houart and Sirenko 2003; Bouchet and Houart 2014) has recently invaded European coasts, probably following massive oyster imports (Pigeot 2000; De Montaudouin & Sauriau 2000; review of Lützen *et al.* 2012). Genetic data suggest that French populations may come from Asia and the United States (Martel *et al.* 2004a). The introduction of *O. inornata* may have important economical consequences, as it is a predator of cultivated mollusks (e.g. oysters, blue mussels, Goulletquer *et al.* 2002). It coexists at several French sites with an indigenous muricid, *O. erinacea* (Linnaeus, 1758), which ranges from the straits of Gibraltar to the Netherlands, and inhabits all British and Mediterranean coasts (Graham 1988). Although *O. erinacea* and *O. inornata* differ in some life history traits (Martel *et al.* 2004c), these muricid gastropods fill similar ecological niches, and may compete for habitat (both species live on hard substrates and drill the shell of bivalves to feed on them; e.g. Lützen *et al.* 2012). Pigeot *et al.* (2000) recorded a decrease in population density of *O. erinacea*, in

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

84 parallel to an increase in numbers of *O. inornata* in Marennes-Oléron (Charente-Maritime,
85 France), between 1997 and 1999 (two years after the invasive was first detected). While the
86 introduction and expansion patterns of *O. inornata* have been investigated in previous studies
87 (Martel *et al.* 2004a; Martel *et al.* 2004b), its ecological impacts on the native *O. erinacea* are
88 poorly known.

89 In previous studies (Martel 2003), seven allozyme loci were analyzed on populations
90 of *Ocenebra erinacea* and *Ocenebra inornata* collected in 7 sites of the French Atlantic coast
91 where the two species live in sympatry. These markers revealed genetic diversity indices
92 systematically weaker within the native *O. erinacea* than within the invasive *O. inornata*. This
93 result was counterintuitive, as (i) the founder effect linked to an introduction event should lead
94 to a low genetic diversity within the populations of the introduced species, (ii) this
95 phenomenon should be all the more marked as the invasion is recent (review of Sakai *et al.*
96 2001). Consequently, a lower genetic diversity within the populations of the exogenous
97 species compared to the populations of the indigenous species was expected. It is thus of
98 importance to test whether the genetic diversity of the indigenous species *O. erinacea* is
99 correlated with the presence of the introduced species *O. inornata* in zones of sympatry.
100 Indeed, *O. inornata* could induce a selective pressure on *O. erinacea* leading to decrease of
101 polymorphism in this local species.

102 Here, we test this hypothesis by sampling *Ocenebra erinacea* from the Atlantic and
103 Mediterranean French coasts, in the presence and absence of *Ocenebra inornata*, and by
104 measuring genetic diversity of the native species using the mitochondrial marker *cox1*. While
105 investigating the genetic effects that the presence of *O. inornata* may have on sympatric
106 populations of *O. erinacea*, we came across a very strong genetic break between Atlantic and
107 Mediterranean populations. This break is detailed and potential biogeographic causes are
108 discussed.

109

110 **Materials and methods**

111

112 Sampling

113 A total of 352 adult specimens of *Ocenebra erinacea* were collected between 1999 and
114 2004 at 15 sites on the French coast, along line transects (<200 m in length). At each site,
115 specimens from different rocks were collected to reduce sampling bias in favor of a particular
116 lineage. The sites were located both within oyster farming zones and unexploited areas (Table
117 1). In order to show a possible impact of the presence of *Ocenebra inornata* on genetic
118 diversity of *O. erinacea*, five locations where the two species live in sympatry and 10 sites
119 free of *O. inornata* have been sampled. The presence of *O. inornata* was assessed by direct
120 observation. After collection, specimens were stored in 95% ethanol before DNA extraction.

121

122 DNA extraction, amplification and sequencing

123 Total DNA was extracted from <15 mg of foot muscle using Dneasy™ Tissue Kit
124 according to the manufacturer's protocol (Qiagen, Germany). Part of the mitochondrial *coxI*
125 gene was PCR-amplified with the HCO2198/LCO1490 primers (Folmer *et al.* 1994), which
126 have proved useful for neogastropod studies (e.g. Zou *et al.* 2011, 2012). Polymorphism at
127 *coxI* is high in *Ocenebra inornata*, a phylogenetically close species (Martel *et al.* 2004a).

128 PCRs were done in 50 µL, with 1X PCR buffer, 1.85 mM MgCl₂, 125 µM dNTPs,
129 0.25 µM of each primer, 1.6 U of Red Hot® DNA Polymerase (ABgene) and about 10 ng of
130 DNA template. The following cycling profile was performed using a MJResearch PTC 100
131 Thermal Cycler: initial 5-min denaturation step at 94°C followed by 40 cycles of 30 sec at

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

94°C, 30 sec at 50°C and 1 min at 72°C, and by a final 5-min extension period at 72°C. PCR products were purified using MultiScreen-PCR MANU03010 plates (Millipore).

Sequencing was done by GenoScreen (Lille - France) using an ABI PRISM® 3730 XL Automated DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA). Sequences were aligned using ClustalX (Thompson *et al.* 1994).

Data analyses.

Genetic analyses aimed at (i) quantifying and comparing genetic diversity among populations, (ii) analyzing the spatial distribution of polymorphism and genetic exchanges among populations, and (iii) studying the evolutionary relationships among populations. Haplotype number H , number of polymorphic sites S , haplotype diversity H_e and average per site nucleotide diversity π (Nei 1987) were calculated for each population using the software DnaSP 5.10.1 (Librado & Rozas 2009).

We tested the null hypothesis of the standard neutral model in Arlequin v.3.5 (Excoffier & Lischer 2010), by calculating the D and F_s statistics, as defined by Tajima (1989) and Fu (1997). When these statistics are significantly different from zero, populations may have undergone purifying selection, a selective sweep and/or expansion (<0), or balancing selection and/or a population decline (>0). Statistical significance was tested by generating 10,000 random samples under the hypothesis of selective neutrality and population equilibrium. These tests were performed for each sampling site separately, and also for pooled sites in presence or absence of the invasive.

The differentiation index Φ_{ST} (Excoffier *et al.* 1992), an estimator of F_{ST} (Wright 1951) calculated from frequency values and distances between haplotypes, was computed with Arlequin v.3.5. The Kimura 2 Parameter (K2P) model of nucleotide substitution was used to

estimate genetic distances, and 10,000 permutations were used to test statistical significance under the null hypothesis of no difference between populations (Excoffier *et al.* 1992).

Finally, a haplotype network was built using the median-joining algorithm implemented in Network 4.6.1.1 (fluxus-engineering.com, Bandelt 1999). This method is one of the most accurate for inferring intra-specific networks in the absence of recombination (Woolley *et al.* 2008).

To help interpret the large genetic divergence observed between Atlantic and Mediterranean specimens of *Ocenebra erinacea*, we looked for mitochondrial *cox1* data from the BOLD database (Ratnasingham & Hebert 2007). However, besides three other BOLD *cox1* sequences from Spanish specimens of *O. erinacea*, we produced the only available mitochondrial sequences for the genus *Ocenebra*. We therefore used *cox1* sequences from six species of the closely-related genus *Nucella* Röding, 1798 (e.g. Pascal 2004) to measure intra-specific and inter-specific genetic distances. *Nucella* and *Ocenebra* are both characterized by a non-planktonic larval development and lay egg capsules on hard substrates (Martel *et al.* 2004c; review by Krug 2011). We used the K2P model of nucleotide substitution (Kimura 1980), widely used in DNA barcoding (Hebert *et al.* 2003; Barrett & Hebert 2005), to measure genetic distances among *cox1* haplotypes.

Results

A 550 bp fragment of *cox1* was sequenced for 352 individuals, and 29 haplotypes were identified (GenBank accession numbers AY995771-99; Popset 63109090). Sequences include 37 polymorphic sites, 20 of which are parsimony informative, and one of which has three character states. No indels were observed (Table 2).

Population-level genetic diversity and demographic stability

Genetic diversity is comparable among Atlantic populations of *Ocenebra erinacea*, but values for the different diversity indices are low compared to other recently-studied marine mollusks. The number of polymorphic sites between two different sequences varies between 0 and 6 among Atlantic populations. Two of these populations (Morbihan and St Quay) are each characterized by a single haplotype, and the 12 other Atlantic sites have no more than five haplotypes (sample sizes provided in Table 1). Consequently, haplotype diversity H_e is low, except for Oléron ($H_e = 0.564$; Table 2). In Fouras, where *Ocenebra inornata* and *O. erinacea* are sympatric, the haplotype and nucleotide diversities are respectively four- and six-fold higher in the invasive drill ($H_e = 0.348$ and $\pi \times 10^{-3} = 0.83$; data from Martel 2003) than in the local one ($H_e = 0.074$ and $\pi \times 10^{-3} = 0.14$).

A single Mediterranean population (Thau) was sampled. The haplotype diversity ($H_e = 0.342$) and the nucleotide diversity ($\pi \times 10^{-3} = 2.52$) are respectively two- and five-fold higher than on the whole of Atlantic populations (Table 2). Moreover, 22 polymorphic sites were found among 37 individuals sampled in Thau, a value considerably higher than the 24 polymorphic sites observed among 315 Atlantic individuals.

Last, haplotype and nucleotide diversities of *Ocenebra erinacea* populations co-occurring with *Ocenebra inornata* were lower ($H_e = 0.247 \pm 0.0535$ and $\pi \times 10^{-3} = 0.57 \pm 0.65$) than for populations located in zones where *O. inornata* was not detected ($H_e = 0.399 \pm 0.3976$, $\pi \times 10^{-3} = 10.04 \pm 5.52$) (Table 2). However, this pattern is entirely due to the higher diversity encountered at Thau; when removing this site from the group of populations that were not found in contact with *O. inornata*, diversity values dropped significantly ($H_e = 0.179 \pm 0.0368$, $\pi \times 10^{-3} = 0.38 \pm 0.51$). Comparing molecular diversity at the site level revealed the same pattern (Welch Two Sample t-test, including Thau, for H_e : $t = -0.83$, $df = 6.22$, $p = 0.44$;

for π : $t = -0.16$, $df = 12.81$, $p = 0.88$. In both cases, results were also non-significant when removing the population of Thau).

Except for one case, the D and F_s statistics were never positive (Table 2). Furthermore, the only slightly positive D value (deviation from zero non significant) was observed when sites where the invasive was absent were pooled, including Thau, and this result was therefore likely influenced by the underlying population structure (see “Genetic differentiation among populations” Results section). There is therefore no supporting evidence that *Ocenebra erinacea* populations exposed to *O. inornata* suffered from a population decline. Some sites exhibited significant negative values of D and F_s , which can be interpreted as signs of purifying selection, selective sweep and/or population expansion. Particularly, the pooled Atlantic sites showed significantly negative values for both tests, regardless of whether the invasive was present or not. These molecular signatures must, however, be interpreted with care, as they might reflect older demographic events.

Genealogical relationships and spatial distribution of haplotypes

Two haplogroups, separated by 18 mutational steps, were observed using the median-joining network (Fig. 1). The first haplogroup (23 haplotypes) was mainly composed of Atlantic specimens, and included one specimen from Thau, characterized by haplotype H26 (separated from other Mediterranean haplotypes by 20 to 22 mutational steps). Haplotypes from this group diverged by ≤ 2 mutations. Haplotype H1 was common (represented in 89% of Atlantic individuals) and central to the Atlantic haplogroup, while the other 22 haplotypes were rare (7 individuals for H3, 3 for H13, 2 for H6, H9, H15, H19 and a single individual for the others) and peripheral to H1. The second haplogroup was strictly composed of Mediterranean individuals. Of six haplotypes from this group, one was common (H21,

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

represented in 82% of Thau individuals), and four were rare (3 individuals for H22 and a single individual for H23-25). Haplotypes from this group diverged by one mutation. The Atlantic samples share no haplotype with the Mediterranean sample (Fig. 2). Except for H1 (present at all sites except Thau), H3 (shared between Loix and Aytré) and H13 (shared between Loix, Aytré and Trébeurden), all haplotypes are private (observed only within one population).

Genetic differentiation among populations

Genetic differentiation among pairs of populations was measured using Φ_{ST} . The population from Thau was significantly differentiated from all other populations. Pairwise Φ_{ST} values ranged between 0.94 and 0.96, corresponding to a substantial genetic differentiation between the Mediterranean and Atlantic populations (Table 3). Inside the Atlantic group, no significant differentiation was observed after sequential Bonferroni correction (lowest corrected alpha level for Atlantic populations: 0.00055).

Levels of intra-specific divergence in *Ocenebra* compared to *Nucella*

Given the differences in haplotype composition and divergence between Mediterranean and Atlantic sites, we investigated whether the genetic distances correspond to intra- or inter-specific divergence by comparing *Ocenebra* to its close relative *Nucella*. The pairwise K2P distance between *Ocenebra erinacea* haplotypes ranged between 0.18 and 4.54% (maximum observed between haplotypes 20 from Loire and 25 from Thau Lagoon). Within *Nucella*, K2P was calculated for 532 sequences and 117 haplotypes distributed among six species, along a 434 bp stretch of *coxI*. Intra-specific distances ranged from 0 to 3.32%, while inter-specific distance ranged from 4.81 to 12.2%, for specimens distributed over 1000 to

2000 km (East and West coasts of North America, respectively; BOLD database). For comparison, Zou et al (2011), analyzing 108 neogastropod *cox1* sequences (same gene region as analysed here; not including *Ocenebra* or *Nucella*) found maximum intra-specific K2P distances of 2.2% and minimum inter-specific distances of 2.1%.

Discussion

Relation between *Ocenebra erinacea* and the invasive species *Ocenebra inornata*

While a decrease in genetic diversity in response to invaders was reported in the past (Kim *et al.* 2003), we did not detect such a pattern among *Ocenebra erinacea* exposed to the invasive species *Ocenebra inornata*. The relatively low polymorphism levels recorded may have hampered our ability to detect genetic effects of the invasive on the native species, and the use of additional molecular markers such as nuclear microsatellites or SNPs might further help detect possible demographic events associated with the presence *O. inornata*. However, this remains to be tested, as even genome-wide scans can fail at detecting recent demographic events and selective pressures. Riquet *et al.* (2013), for instance, used AFLPs to compare native and invasive populations of the marine mollusk *Crepidula fornicata*. They reported little genetic differentiation among these populations, and detected no F_{ST} outliers out of 344 tested loci. An alternative hypothesis explaining the apparent absence of genetic effects of the invasive on the native is that the competitive and selective pressures inflicted on *O. erinacea* are too low to have genetic effects (e.g. Wittmann *et al.* 2013). Finally, deviations from neutrality observed for pooled Atlantic sites suggest selection could have shaped the current genetic diversity and could have blurred signatures of demographic processes.

Ocenebra inornata was first documented on the French Atlantic coast in 1995 (De Montaudouin & Sauriau 2000, Pigeot *et al.* 2000), and the specimens of *Ocenebra erinacea*

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

used in this study were collected between 1999 and 2004. The introduction of *O. inornata* may have been too recent at the time of sampling for genetic consequences on the native species to be detectable. About ten years later, the distributional landscape of *O. inornata* on Atlantic coasts have significantly changed, and the invasive is now found as far north as the entrance of the Baltic Sea (Lützen *et al.* 2012). A new survey of the genetic diversity of *O. erinacea* may today unfold the genetic consequences of the invasion by *O. inornata*, and this study therefore provides a snapshot in time that may help better understand the temporal dynamics of loss of genetic diversity. In addition to sampling in the field, we searched for *Ocenebra* specimens in the collections of the Museum national d'Histoire naturelle in Paris (France), in order to look for genetic diversity in *O. erinacea* specimens collected prior to, or soon after the invasion by *O. inornata* (MNHN voucher numbers IM-2008-7101, IM-2008-7102, IM-2008-7103). Unfortunately, we were not able to amplify the *cox1* marker from these specimens.

Remarkably low genetic diversity of *Ocenebra erinacea* populations

Mitochondrial genetic diversity, as measured using part of *cox1*, was low relative to what was observed in other marine mollusks. Overall, *Ocenebra erinacea* haplotype and nucleotide diversities were (disregarding the sample from Thau, see below) $H_e = 0.18-0.25$ and $\pi \times 10^{-3} = 0.38-0.57$ (Table 2). Comparatively, $H_e = 0.684$ and $\pi \times 10^{-3} = 2.25$ for *Ocenebra inornata* in its natural range (data from Martel *et al.* 2004a), $H_e = 0.734$ and $\pi \times 10^{-3} = 14.78$ in the gastropod *Cyclope neritea* (Simon-Bouhet *et al.* 2006), and $H_e = 0.720$ and $\pi \times 10^{-3} = 89.84$ in the bivalve *Macoma balthica* (Becquet *et al.* 2012).

The low genetic diversity observed at *cox1* was consistent with the low diversity observed using allozymes: Martel (2003) reported that the number of alleles N_{all} and the

observed heterozygosity H_o characterizing the Atlantic populations of *Ocenebra erinacea* are respectively two to four times, and twenty to thirty times lower ($N_{all} = 1.1 \pm 0.1$; $H_o = 0.01 \pm 0.01$; mean $\pm$ SD) than in other marine gastropods sampled in their native range, such as *Bedevea hanleyi* ($N_{all} = 2.2 \pm 0.1$, $H_o = 0.30 \pm 0.02$; Hoskin 2000), *Drupella* sp. ($N_{all} = 2.3 \pm 1.0$, $H_o = 0.25$; Johnson & Cumming 1995), and *Littorina striata* ($N_{all} = 4.2 \pm 1.0$, $H_o = 0.18 \pm 0.17$; De Wolf *et al.* 2000). Congruent patterns across mitochondrial and allozyme markers thus suggest a low genetic diversity in these populations rather than an absence of variability at *cox1*. Still, our sampling remains restricted compared to the native range of the species, and additional monitoring may reveal new patterns of genetic diversity.

Genetic diversity and connectivity among populations

The genetic diversity of the Mediterranean population was among the highest (Table 2). The oyster farmers of the Thau lagoon, one of the main shell farming areas of the French Mediterranean coasts, carry out commercial exchanges with distant production sites, and *Ocenebra erinacea* is likely to be transferred during these exchanges. In fact, the morphological survey done by Berrou *et al.* (2004) evidenced exchanges from Oléron Island to Thau Lagoon (this is congruent with the observation of the Atlantic haplotype H26 being observed at Thau; Figs. 1-2). In our study, the high genetic diversity observed in Thau Lagoon could be the result of the introduction of Mediterranean specimens imported from other production sites such as Oléron. As no Mediterranean site was sampled other than Thau Lagoon, the artificial mixing induced by shellfish exchanges cannot be further evaluated here.

Alternatively, the difference in genetic diversity observed between the Atlantic and the Mediterranean population of Thau lagoon could be explained by historical and biogeographical factors, and/or selection. *Ocenebra erinacea* is not well adapted to cold water

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

and Belgium currently constitutes the northern limit of its natural range (Graham 1988). Consequently, the species may have found, as other marine species (e.g. Nikula & Väinölä 2003, Ladhar-Chaabouni *et al.* 2010), a refugium on the Iberian coast or in the Mediterranean basin during past glaciations. *O. erinacea* may have disappeared from the French Atlantic coasts during the Würm Glacial period (115,000 to 10,000 years BP) but survived on the coasts of the Iberian peninsula, which is known as one of the major Pleistocene refugia (Gómez & Lunz 2007). At the end of this climatic crisis, a reduced number of individuals from southern refuges may have reached northern coasts. Maggs *et al.* (2008), reviewing molecular signatures of glacial refugia on marine species, made predictions of low genetic and haplotype diversity in northern regions previously covered by ice sheets, and comparatively high diversity in refugial southern regions (and see Hewitt 1996). These predictions are generally met for *O. erinacea*, but additional sampling from the Iberian peninsula and the Mediterranean Sea would be necessary to further characterize the historical biogeography of this species. Given *O. erinacea*'s maladaptation to cold-water, another possibility is that selection (either purifying selection, or selective sweeps) linked to differences in water temperature between Thau and the Atlantic sites produced the observed patterns of genetic diversity. The negative Tajima's *D* and Fu's *F_s* observed for the Atlantic population (in absence and presence of the invasive) would support this scenario.

One potential consequence of biogeographic divergence between Atlantic and Mediterranean populations is the emergence of new species (Hewitt 1996, 2004). Recently, Salicini *et al.* (2013) have shown that in the bat *Myotis nattereri*, a complex of four cryptic species exist in the Western Palearctic region (central and southern Europe, northwestern Maghreb), each species coinciding with a glacial refugium. In *Ocenebra erinacea*, inter-clade divergence overlaps with the inter-specific distances observed in the closely-related genus *Nucella* (even though the geographical distances separating *Nucella* specimens were greater

than the distances separating *O. erinacea* specimens; see Bergsten *et al.* 2012). In addition, *O. erinacea* specimens from the Atlantic and Mediterranean can readily be distinguished using morphology, and the morphological distance between Atlantic and Mediterranean *O. erinacea* is comparable to what is observed between *O. erinacea* and *Ocenebra brevirobusta* Houart 2000 (Berrou *et al.* 2004). It is therefore possible that the Atlantic and Mediterranean clades sampled for this study belong to groups in incipient stages of speciation, or even undescribed species.

Acknowledgements

We thank Stéphane Robert (IFREMER, La Tremblade, France), Yvonne Pascal, Michel Pascal (INRA Rennes, France), François Siorat (LPO), Armel Deniau (LPO), Marie-Gabrielle Vitre, Paul Wendling, Françoise Dreau, Christian Bussière, Isabelle Kuhne, Jean-Paul Egreteau, José Simone Guézou, Sandrine Sourisseau, Xavier de Montaudouin (University of Bordeaux, France), Pierre-Guy Sauriau (IFREMER, L'Houmeau, France) for their help with field sampling, Jacques Pigeot for his insights and help with identifications, Stéphane Robert (IFREMER, La Tremblade, France), Philippe Gouletquer (IFREMER, La Tremblade, France) and Yves Pichot (IFREMER, Sète, France) for information on shellfish transfers in France, Pierre Lozouet at the Muséum national d'Histoire naturelle (Paris, France) for granting access to the *Ocenebra* collection, and Alice Saunier for her help in the lab. This work was funded by the Conseil Général de Charente-Maritime and the Ministère de l'Ecologie et du Développement Durable (Program INVABIO no. D4E/SRP/01115). Salaries for EP & AV were covered by a grant to the Poitou-Charentes region (Contrat de Projet État-Région 2007-2013), by a grant from the Fond Européen de Développement Régional (EP), and by Actions Internationales et Rayonnement of the University of La Rochelle (AV). We thank the three

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

anonymous reviewers and the editor, whose comments significantly improved the manuscript.
This work is dedicated to the memory of Michel Pascal.

References

Amano, K., G.J. Vermeij (1998) Taxonomy and evolution of the genus *Ocenebrellus* (Gastropoda: Muricidae) in Japan. *Paleontological Research*, **2**, 199-212.

Bandelt, H., P. Forster, et al. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37-48.

Barrett, R., P.D.N. Hebert (2005) Identifying spiders through DNA barcodes. *Canadian Journal of Zoology*, **83**, 481–491.

Becquet, V., B. Simon-Bouhet, et al. (2012) Glacial refugium versus range limit: conservation genetics of *Macoma balthica*, a key species in the Bay of Biscay (France). *Journal of Experimental Marine Biology and Ecology*, **432-433**, 73-82.

Bergsten, J., D.T. Bilton, T. Fujisawa, et al. (2012) The effect of geographical scale of sampling on DNA barcoding. *Systematic Biology*, **61**, 851–869.

Berrou, V., D. Merle, et al. (2004) Comparative morphology of Pliocene, Quaternary and Recent shells of *Ocenebra erinaceus* (Linnaeus, 1758) and *O. brevirobusta* Houart, 2000 (Mollusca, Muricidae, Ocenebrinae): reflections on the intra- and interspecific variations. *Geodiversitas*, **26**(2), 263-295.

Bouchet, P., R. Houart (2014). *Ocenebra inornata* (Récluz, 1851). Accessed through: World Register of Marine Species at <http://www.marinespecies.org/aphia.php?p=taxdetails&id=403745> on 2014-05-02.

Carlton, J.T. (1989) Man’s role in changing the face of the ocean: biological invasions and implications for conservation of near shore environments. *Conservation Biology* **3**: 265-273.

- 1
2
3 400 Carlton, J.T. (1992) Introduced marine and estuarine mollusks of North America: an end-of-
4
5 401 the-20th-century perspective. *Journal of Shellfish Research*, **11**, 489-505.
6
7 402 Choe, B.L., J.K. Park (1997) Description of Muricid species (Gastropoda: Neogastropoda)
8
9 403 collected from the coastal areas of south Korea. *Journal of Biological Science*, **1**, 281-
10
11 404 296.
12
13 405 De Montaudouin, X., P.-G. Sauriau (2000) Contribution to a synopsis of marine species
14
15 406 richness in the Pertuis Charentais Sea with new insights in soft-bottom macrofauna of
16
17 407 the Marennes-Oléron Bay. *Cahiers de Biologie Marine*, **41**, 188-222.
18
19 408 De Wolf, H., R. Verhagen, et al. (2000) Large scale population structure and gene flow in the
20
21 409 planktonic developing periwinkle *Littorina striata* (Mollusca: Gastropoda), in
22
23 410 Macronésia. *Journal of Experimental Marine Biology and Ecology*, **246**, 69-83.
24
25 411 Elton, C. (1958). *The ecology of invasions by animals and plants*. University of Chicago
26
27 412 Press.
28
29 413 Excoffier, L., H.E.L. Lischer (2010) Arlequin suite ver 3.5: A new series of programs to
30
31 414 perform population genetics analyses under Linux and Windows. *Molecular Ecology*
32
33 415 *Resources*, **10**, 564-567.
34
35 416 Excoffier, L., P.E. Smouse, et al. (1992) Analysis of Molecular Variance Inferred From
36
37 417 Metric Distances Among DNA Haplotypes: Application to Human Mitochondrial DNA
38
39 418 Restriction Data. *Genetics*, **131**, 479-491.
40
41 419 Folmer, O., M. Black, et al. (1994) DNA primers for amplification of mitochondrial
42
43 420 cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine*
44
45 421 *Biology and Biotechnology*, **3**, 294-299.
46
47 422 Frankham, R. (1995) Conservation genetics. *Annual Review of Genetics*, **29**, 305-327.
48
49 423 Fu, Y-X. (1997) Statistical tests of neutrality of mutations against population growth,
50
51 424 hitchhiking and background selection. *Genetics*, **147**, 915-925.
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

425 Garton, D.W., W.R. Haag (1991) Heterozygosity, shell length and metabolism in the
426 European mussel, *Dreissena polymorpha*, from a recently established population in lake
427 Erie. *Comparative Biochemistry and Physiology*, **99**, 45-48.

428 Gouletquer, P., G. Bachelet, et al. (2002) Open Atlantic coast of Europe - a century of
429 introduced species into French waters. In: E. Leppäkoski, S. Gollash & S. Olenin (Eds).
430 *Invasive species of Europe - Distribution, impact and management*. Kluwer Academic
431 Publishers, Dordrecht / Boston / London.

432 Gómez, A., D.H. Lunt (2007) Refugia within Refugia: Patterns of Phylogeographic
433 Concordance in the Iberian Peninsula. In: S. Weiss, N. Ferrand (Eds). *Phylogeography of*
434 *Southern European Refugia*. Netherlands: Springer, volume III, pp. 155-188.

435 Graham, A. (1998). *Molluscs: Prosobranchs and Pyramidellid Gastropods: Keys and Notes*
436 *for the Identification of the Species*. Linnean Society of London.

437 Hebert, P.D.N., A. Cywinska, et al. (2003) Biological identifications through DNA barcodes.
438 *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270**, 313–321.

439 Hedgecock, D., L. Jing-Zhong, S. DeCola, C.D. Haudenschield, E. Meyer, D.T. Manahan, B.
440 Bowen (2007) Transcriptomic analysis of growth heterosis in larval Pacific oysters
441 (*Crassostrea gigas*). *Proceedings of the National Academy of Sciences of the United States*
442 *of America*, **104**, 2313-2318.

443 Hewitt, G.W. (1996) Some genetic consequences of ice ages, and their role in divergence and
444 speciation. *Biological Journal of the Linnean Society*, **58**, 247-276.

445 Hewitt, G.M. (2004) Genetic consequences of climatic oscillations in the quaternary.
446 *Philosophical transactions of the Royal Society of London. Series B, Biological Sciences*,
447 **359**, 183–195.

- 448 Hoskin, M.G. (2000) Effects of the east Australian current on the genetic structure of a direct
449 developing muricid snail (*Bedevea hanleyi*, Angas): variability within and among local
450 populations. *Biological Journal of the Linnean Society*, **69**, 245-262.
- 451 Houart, R., B.I. Sirenko (2003) Review of the Recent species of *Ocenebra* Gray, 1847
452 and *Ocinebrellus* Jousseaume, 1880 in the Northwestern Pacific. *Ruthenica*, **13**, 53-74.
- 453 Johnson, M.S., R.L. Cumming (1995) Genetic distinctness of three widespread and
454 morphologically variable species of *Drupella* (Gastropoda: Muricidae). *Coral Reefs*, **14**,
455 71-78.
- 456 Kim, M.S., S.D. Brunsfeld, et al. (2003) Effect of white pine blister rust (*Cronartium ribicola*)
457 and rust-resistance breeding on genetic variation in western white pine (*Pinus*
458 *monticola*). *Theoretical and Applied Genetics*, **106**, 1004-1010.
- 459 Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions
460 through comparative studies of nucleotide-sequences. *Journal of Molecular Evolution*, **16**,
461 111-120.
- 462 Krug, P. (2011) Patterns of speciation in marine gastropods: a review of the phylogenetic
463 evidence for localized radiations in the sea. *American Malacological Bulletin*, **29**, 169-186.
- 464 Ladhar-Chaabouni, R., A. Hamza-Chaffai, et al. (2010) A pilot study of genetic differentiation
465 between two phenotypes of a Mediterranean population of the bivalve *Cerastoderma*
466 *glaucum* and genetic discrimination with other *Cerastoderma glaucum* and
467 *Cerastoderma edule* populations outside the Mediterranean. *Marine Ecology*, **31**, 355-
468 363.
- 469 Lande, R. (1995) Mutation and conservation. *Conservation Biology*, **9**, 782-791.
- 470 Launey, S., D. Hedgecock (2001) High genetic load in the Pacific oyster *Crassostrea gigas*.
471 *Genetics*, **159**, 255-265

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

472 Librado, P., J. Rozas (2009) DnaSP v5: A software for comprehensive analysis of DNA
473 polymorphism data. *Bioinformatics*, **25**, 1451-1452.

474 Lockwood, J.L., M.F. Hoopes, M.P. Marchetti (2007) *Invasion Ecology*. Blackwell Publishing,
475 Oxford, UK. 304 pp.

476 Lützen, J., M. Faasse, et al. (2012) The Japanese oyster drill *Ocenebrellus inornatus* (Récluz,
477 1851) (Mollusca, Gastropoda, Muricidae), introduced to the Limfjord, Denmark.
478 *Aquatic Invasions*, **7**(2), 181-191.

479 Mack, R.N., D. Simberloff, et al. (2000) Biotic invasions: causes, epidemiology, global
480 consequences and control. *Ecological Application*, **10**, 690-710.

481 Maggs, C.A., R. Castilho, et al. (2008) Evaluating signatures of glacial refugia for North
482 Atlantic benthic marine taxa. *Ecology*, **89**, S108-S122.

483 Martel C. (2003) *Invasions biologiques et perturbations anthropiques des écosystèmes*
484 *littoraux : source, profil d'expansion et impacts d'un gastéropode marin*, *Ocenebrellus*
485 *inornatus*, *introduit accidentellement sur les côtes atlantiques françaises*. Doctoral thesis,
486 Université de La Rochelle. 122 pp & Annexes.

487 Martel, C., D. Bourguet, et al. (2004b) Invasion by the marine gastropod *Ocenebrellus*
488 *inornatus* in France. II. Expansion along the Atlantic coast. *Marine Ecology Progress*
489 *Series*, **273**, 163-172.

490 Martel, C., J.M. Guarini, et al. (2004c) Invasion by the marine gastropod *Ocenebrellus*
491 *inornatus* in France. III. Comparison of biological traits with the resident species
492 *Ocenebra erinacea*. *Marine Biology*, **146**, 93-102.

493 Martel, C., F. Viard, et al. (2004a) Invasion by the marine gastropod *Ocenebrellus inornatus*
494 in France. I. Scenario for the source of introduction. *Journal of Experimental Marine*
495 *Biology and Ecology*, **305**, 155-170.

- 496 Mitton, J.B., M.C. Grant (1984) Associations among protein heterozygosity, growth rate and
497 developmental homeostasis. *Annual review of Ecology and Systematics*, **15**, 479-499.
- 498 Mooney, H.A., E.E. Cleland (2001) The evolutionary impact of invasive species. *Proceedings*
499 *of the National Academy of Sciences of the United States of America*, **98**, 5446-5451.
- 500 Nei M. (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- 501 Nikula, R., R. Väinölä (2003) Phylogeography of *Cerastoderma glaucum* (Bivalvia:
502 Cardiidae) across Europe: a major break in the Eastern Mediterranean. *Marine Biology*,
503 **143**, 339-350.
- 504 Pante, E., B. Simon-Bouhet (2013) marmap: A Package for Importing, Plotting and Analyzing
505 Bathymetric and Topographic Data in R. *PloS One*, **8**(9), e73051.
- 506 Parmesan C., G. Yohe (2003) A globally coherent fingerprint of climate change impacts
507 across natural systems. *Nature*, **421**, 37-42.
- 508 Pascal P.-Y. (2004) *Rôle des espèces autochtones dans les processus d'invasions biologiques*
509 *marines: l'exemple du perceur invasif Ocinebrellus inornatus et du perceur autochtone*
510 *Ocenebra erinacea sur les côtes françaises*. Masters Thesis, Université de La Rochelle,
511 47pp.
- 512 Pigeot, J., P. Miramand, et al. (2000) Présence d'un nouveau prédateur de l'huître creuse,
513 *Ocinebrellus inornatus* (Recluz, 1851), dans le bassin conchylicole de Marennes-
514 Oléron. *Comptes-rendus de l'Académie des Sciences de Paris*, **323**, 697-703.
- 515 Ratnasingham, S., P.D.N. Hebert (2007) BOLD : The Barcode of Life Data System BOLD:
516 The Barcode of Life Data System (<http://www.barcodinglife.org>). *Molecular Ecology*
517 *Notes*, **7**, 355-364.
- 518 Riquet, F., C. Daguin-Thiébaud, et al. (2013) Contrasting patterns of genome-wide
519 polymorphism in the native and invasive range of the marine mollusc *Crepidula*
520 *forficata*. *Molecular Ecology*, **7**, 355-364.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

521 Ruiz, G.M., T.K. Rawlings, et al. (2000) Global spread of microorganisms by ships: ballast
522 water discharged from vessels harbours a cocktail of potential pathogens. *Nature*, **408**,
523 49-50.

524 Sakai, A.K., F.W. Allendorf, et al. (2001) The population biology of invasive species. *Annual*
525 *Review of Ecology and Systematics*, **32**, 305-332.

526 Salicini I., C. Ibañez , et al. (2013) Deep differentiation between and within Mediterranean
527 glacial refugia in a flying mammal, the *Myotis nattereri* bat complex. *Journal of*
528 *Biogeography*, **40**, 1182–1193.

529 Seebens H., M.T. Gastner, et al. (2013) The risk of marine bioinvasion caused by global
530 shipping. *Ecology Letters*, **6**, 782-790.

531 Simon-Bouhet, B., P. Garcia-Meunier, et al. (2006) Multiple introductions promote range
532 expansion of the mollusc *Cyclope neritea* (Nassariidae) in France: evidence from
533 mitochondrial sequence data. *Molecular Ecology*, **15**, 1699-1711.

534 Strauss, S.Y., J.A. Lau, et al. (2006) Evolutionary responses of natives to introduced species:
535 what do introductions tell us about natural communities? *Ecology Letters*, **9**, 357-374.

536 Tajima, F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA
537 polymorphism. *Genetics*, **123**(3), 585-595.

538 Thompson, J.D., T.J. Gibson, et al. (1997) The ClustalX windows interface: flexible strategies
539 for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Research*,
540 **24**, 4876-4882.

541 Wittmann, M.J., M. Hutzenthaler, et al. (2013) Ecological and genetic effects of introduced
542 species on their native competitors. *Theoretical Population Biology*, **84**, 25-35.

543 Woolley, S.M., D. Posada, et al. (2008) A comparison of phylogenetic network methods using
544 computer simulation. *PloS One*, **3**, e1913.

- 1
2
3 545 Wright, S. (1951) The genetical structure of populations. *Annals of Human Genetics*, **15**, 323-
4
5 546 354.
6
7 547 Zou, S., Q. Li, L. Kong, H. Yu, X. Zheng (2011) Comparing the Usefulness of Distance,
8
9 548 Monophyly and Character-Based DNA Barcoding Methods in Species Identification: A
10
11 549 Case Study of Neogastropoda. *PLoS One*, **6**, e26619.
12
13 550 Zou, S., Q. Li, L. Kong (2012) Multigene barcoding and phylogeny of geographically
14
15 551 widespread muricids (Gastropoda: Neogastropoda) along the coast of china. *Marine*
16
17 552 *Biotechnology*, **14**, 21–34.
18
19 553 Zouros, E. (1993) Associative overdominance evaluating the effects of inbreeding and linkage
20
21 554 disequilibrium. *Genetica*, **89**, 35-46.
22
23 555
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1. *Ocenebra erinacea* collection sites (listed from North to South, following the coastline), site name abbreviations (Ab., as used in Figure 2), number of *O. erinacea* specimens collected and sequenced (n), geographical coordinates, year of collection, and characteristics of the locations: presence (+) or absence (-) of oyster farms and of *O. inornata*.

Location	Ab.	n	Latitude	Longitude	Year of collection	Shellfish area	Presence of <i>O. inornata</i>
Blainville	Bl	31	49°03' N	1°36' W	2003	+	-
Chaussey	Ch	25	48°52' N	1°48' W	2003	+	-
Saint Malo	SM	22	48°39' N	2°01' W	2004	-	-
Saint Quay	SQ	23	48°39' N	2°50' W	2004	-	-
Trébeurden	Tr	24	48°48' N	3°35' W	2004	-	-
Crozon	Cr	23	48°17' N	4°27' W	2004	-	-
Le Croisic	LC	22	47°18' N	2°31' W	2004	-	-
Morbihan	Mo	12	47°33' N	2°51' W	2004	-	-
Bourgneuf	Bo	24	47°01' N	2°01' W	2003	+	+
Loix	Lo	28	46°13' N	1°24' W	2003	+	+
Aytré	Ay	20	46°06' N	1°07' W	2003	+	+
Fouras	Fo	27	46°00' N	1°07' W	2004	+	+
Oléron	Ol	13	45°53' N	1°10' W	2004	+	+
Arcachon	Ar	21	44°40' N	1°12' W	1999	+	-
Thau	Th	37	43°24' N	3°35' W	1999	+	-

Table 2. Molecular diversity of populations and results of the neutrality tests. The number of segregating sites (S), the number of haplotypes (H), the haplotype diversity ($H_e \pm$ one standard deviation), and the nucleotide diversity ($\pi \pm$ one standard deviation) are given for each sampling site, site groups in presence and in absence of the invasive (with and without the Mediterranean population of Thau), and for the entire data set. Sites where *Ocenebra inornata* was present are indicated by an asterisk. For neutrality tests of Tajima and Fu, statistical significance after sequential Bonferroni correction is indicated with an asterisk.

Sampling sites	S	H	$H_e \pm SD$	$\pi \pm SD (x10^{-3})$	Tajima's D	Fu's F_s
Blainville	1	2	0.065 ± 0.059	0.12 ± 0.28	-1.14	-1.24*
Chaussey	3	4	0.230 ± 0.110	0.44 ± 0.58	-1.73	-3.08*
Saint Malo	1	2	0.091 ± 0.081	0.17 ± 0.34	-1.16	-0.96
Saint Quay	0	1	0	0	0	0
Trébeurden	5	5	0.377 ± 0.122	0.90 ± 0.89	-1.83	-2.80*
Crozon	2	3	0.316 ± 0.118	0.60 ± 0.70	-0.86	-0.87
Le Croisic	4	4	0.333 ± 0.124	0.81 ± 0.84	-1.67	-1.74
Morbihan	0	1	0	0	0	0
Bourgneuf *	2	2	0.083 ± 0.075	0.30 ± 0.47	-1.51	-0.19
Loix *	5	5	0.270 ± 0.109	0.65 ± 0.72	-2.01*	-3.57*
Aytré *	4	4	0.363 ± 0.131	0.89 ± 0.89	-1.64	-1.61
Fouras *	1	2	0.074 ± 0.067	0.14 ± 0.30	-1.15	-1.12
Oléron *	2	3	0.564 ± 0.112	1.12 ± 1.06	-0.13	-0.17
Arcachon	1	2	0.095 ± 0.084	0.17 ± 0.35	-1.16	-0.92
Thau	22	6	0.342 ± 0.098	2.52 ± 1.76	-2.50*	-0.42
Sites in presence of invasive	11	9	0.247 ± 0.0535	0.57 ± 0.65	-2.10*	-9.42*
Sites in absence of invasive	31	22	0.399 ± 0.3976	10.04 ± 5.52	0.22	-0.06
Sites in absence of invasive (without Thau)	16	16	0.179 ± 0.0368	0.38 ± 0.51	-2.38*	< -10*
All populations	37	29	0.356 ± 0.0325	7.63 ± 4.20	-0.81	-4.97

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

Table 3. Pairwise Φ_{ST} values calculated using the Kimura 2 Parameter model of nucleotide substitution. Only pairwise comparisons involving the population of Thau are statistically significant after sequential Bonferroni correction.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Blainville	0														
2. Chausey	0.0048	0													
3. Saint Malo	0.00242	0.00244	0												
4. Saint Quay	0.01001	0.00356	0.00207	0											
5. Trebeurden	0.02194	0.01045	0.00921	0.01207	0										
6. Crozon	0.05037	0.02772	0.03452	0.04529	0.02682	0									
7. Le Croisic	0.03001	0.01442	0.01577	0.02118	0.01654	0.01135	0								
8. Morbihan	0.03708	0.03371	0.03067	0	0.02156	0.00476	0.01543	0							
9. Bourgneuf	0.00458	0.00021	0.00102	0.00181	0.01083	0.0316	0.01598	0.03274	0						
10. Loix	0.00242	-0.0008	0.00559	0.00747	0.00258	0.02217	0.012	-0.0358	0.01337	0					
11. Aytré	0.03811	0.01838	0.02156	0.0293	0.00956	0.03281	0.02045	0.01049	0.00555	0.01096	0				
12. Oleron	0.28743	0.18725	0.23275	0.28709	0.1342	0.16138	0.13706	0.18898	0.15611	0.12163	0.04004	0			
13. Fouras	0.0004	0.00162	0.00096	0.00611	0.01656	0.04351	0.02394	0.03502	0.00196	0.00096	0.03104	0.26425	0		
14. Arcachon	0.0032	0.00323	0.00014	0.00443	0.00763	0.03266	0.01404	0.02941	0.00153	0.00658	0.01955	0.22606	0.00145	0	
15. Thau	0.96208	0.95559	0.95617	0.95856	0.94988	0.95236	0.95001	0.94928	0.95614	0.9547	0.9484	0.94167	0.95959	0.95541	0

Fig. 1. Median-joining haplotype network. Each circle represents a haplotype, which frequency is proportional to circle diameter (legend: bottom right). Distances between haplotypes are proportional to the number of mutation events (see text). The Atlantic haplogroup contains one Mediterranean population, represented by haplotype H26 (marked with **).

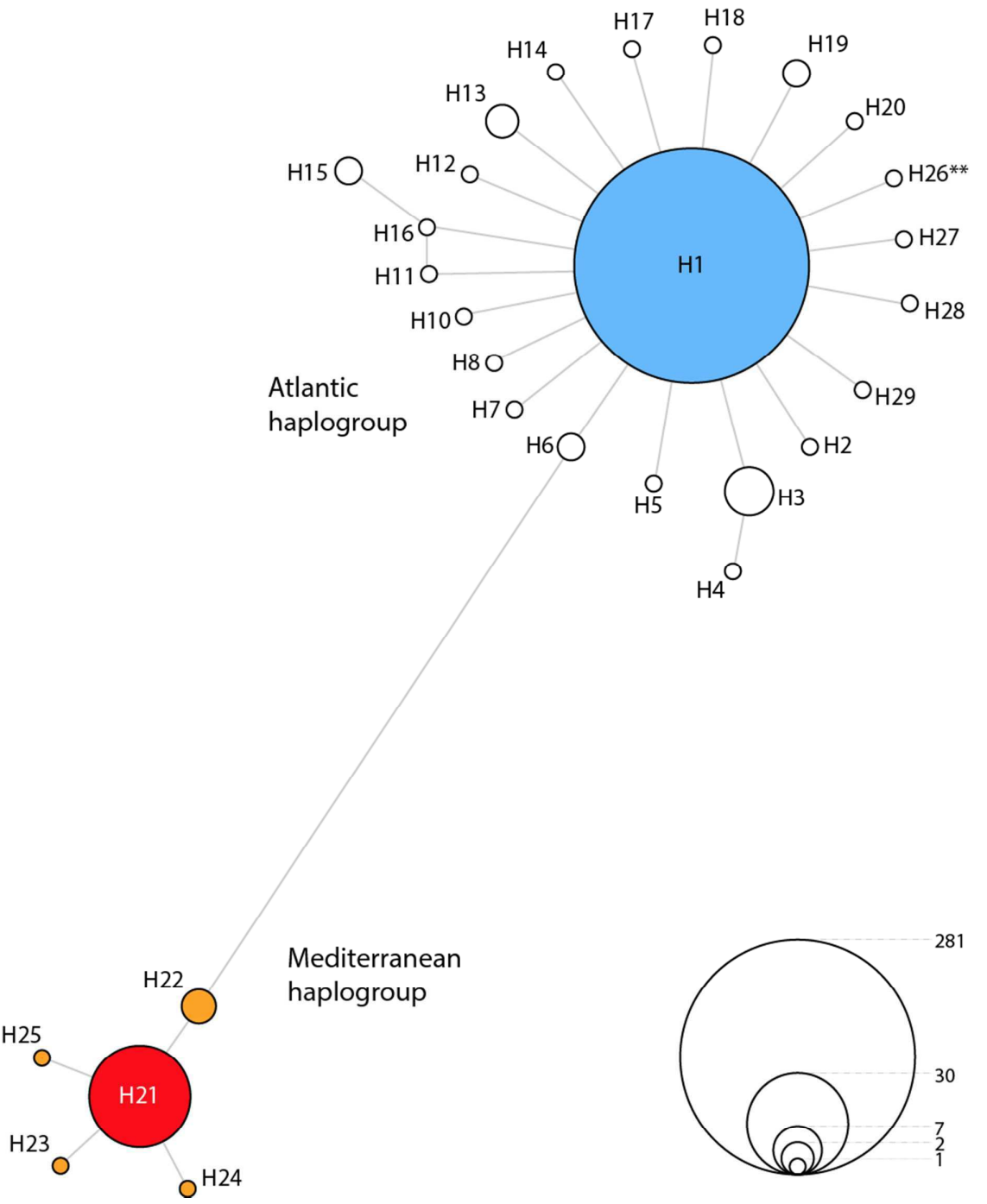
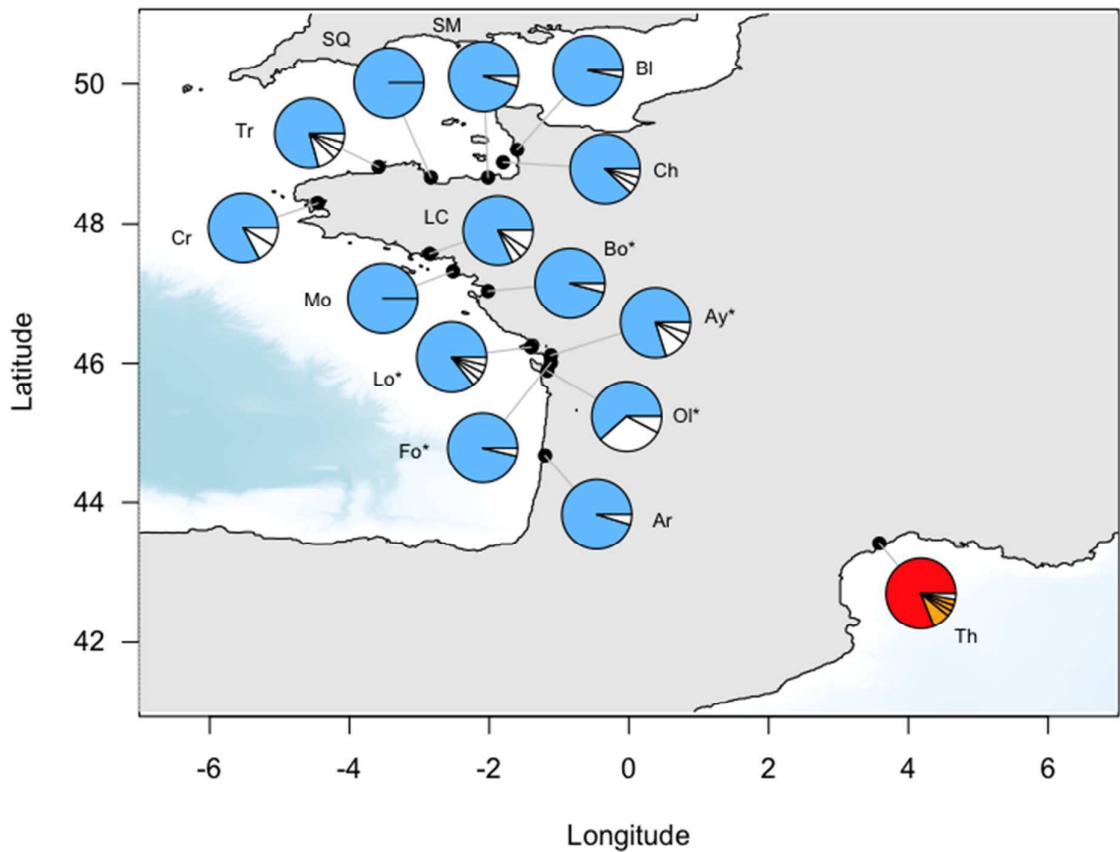


Fig. 2. Distribution of haplotype frequencies along the French coast. Abbreviations for site names are detailed in Table 1. Haplotype colors correspond to the colors used in Figure 1. The stations where both *Ocenebra erinacea* and *Ocenebra inornata* were observed are marked with an asterisk. Map constructed with R package marmap (Pante & Simon-Bouhet 2013).



Evaluating the genetic effects of the invasive *Ocenebrelluscenebra inornatus* on the native oyster drill *Ocenebra erinacea*

Eric Pante¹, Pierre-Yves Pascal², Vanessa Becquet¹, Amélia Viricel¹, Benoit Simon-Bouhet¹, and Pascale Garcia¹

¹ Littoral, Environnement et Sociétés (LIENSs) UMR 7266 CNRS – Université de La Rochelle, 2 rue Olympe de Gouges, 17000 La Rochelle, France.

² Systématique-Adaptation-Evolution, Equipe Biologie de la Mangrove, Laboratoire de Biologie Marine, UMR 7138 CNRS-MNHN-UPMS, Université des Antilles et de la Guyane, BP 592 F-97159 Pointe-à-Pitre cedex, Guadeloupe, France

Keyword: *Ocenebra erinaceus*; *Ocenebrellus inornatus*; haplotype diversity; evolutionary response; evolutionary ecology; invasive species; glacial refugium; cryptic species

1
2
3
4
5
6
7 16 **Abstract**
8
9 17
10
11 18 Studies focusing on the effect of invasive species on the genetic diversity of native marine
12
13 19 invertebrates remain scant. Here we report diversity among French populations of the
14
15 20 intertidal gastropod *Ocenebra erinacea* (Linnaeus, 1758) sampled in the presence and absence
16
17 21 of the invasive ~~*Ocenebrellus inornatus*~~*Ocenebra inornata* (Recluz, 1851). Between 1999 and
18
19 22 2004 a total of 352 individuals of *O. erinacea* were collected from 15 sites, five of which in
20
21 23 the presence of the invasive, and genotyped at the mitochondrial locus *cox1*. No statistical
22
23 24 difference was observed between polymorphism levels recorded within native populations
24
25 25 exposed to the invasive, compared to populations sampled in the absence of ~~*O. inornatus*~~*O.*
26
27 26 *inornata*. No sign of native population ~~bottleneck-decline~~ was detected in response to the
28
29 27 invader. While significant shifts in native *O. erinacea* population sizes were previously
30
31 28 reported in the literature, genetic effects may take longer to accumulate, ~~or may be~~
32
33 29 ~~undetectable without a larger panel of genetic markers~~~~or may be detectable using a larger~~
34
35 30 ~~panel of genetic markers~~. In contrast, large genetic distances and significant population
36
37 31 differentiation were recorded between Atlantic and Mediterranean *O. erinacea* samples,
38
39 32 suggesting that these populations have distinct evolutionary histories. Comparison of genetic
40
41 33 divergence within the closely-related genus *Nucella* suggests that the Atlantic populations and
42
43 34 the Mediterranean *O. erinacea* populations from Thau Lagoon may belong to different species
44
45 35 or subspecies.
46
47 36
48
49
50
51
52
53
54
55
56
57
58
59
60

Introduction

Natural movements of species ranges on the surface of the world (expansions, regressions, displacements) play a considerable role in the evolution of species. Most of the time, these phenomena are progressive and marked by the tempo of geological processes (e.g. Hewitt 1996). However, ~~for a~~ during the last few decades a growing number of species has undergone changes in their natural range due to both ~~global~~ changes at ~~the planetary~~ global scale (e.g. Parmesan & Yohe 2003) and artificial transfers due to human activities (e.g. Carlton 1989; Seebens *et al.* 2013).

The number of biological invasions has strongly increased during the last decades (e.g. Mack *et al.* 2000; Mooney & Cleland, 2001; Ruiz *et al.* 2000). In the marine environment, these phenomena are mainly due to aquaculture and especially to shellfish farming, which represents a major cause of introduction, intentional or not, of exogenous species (Elton 1958; Carlton 1992).

Apart from potentially important economic consequences, the arrival of these introduced species can also cause serious ecological impacts on local fauna. Introduced species are likely to decrease the abundance of indigenous species, excluding them from part of their distributional area or even causing their extinction by modifying invaded habitats, hybridizing with native species, exchanging pathogens, preying on them, or competing with them (e.g. Lockwood *et al.* 2007 and references therein). Moreover, when they exert strong selective pressures, introduced species can also reduce the genetic diversity of native populations (Kim *et al.* 2003; Wittman *et al.* 2013).

Such impacts, although poorly known (e.g. Strauss *et al.* 2006), may have heavy ecological consequences since adaptive potential depends on the genetic diversity of a

1
2
3
4
5
6
7 61 population. Reductions in genetic diversity are generally considered detrimental (e.g.
8
9 62 Frankham 1995; Lande 1995; Strauss *et al.* 2006) and ~~may lead~~might contribute to extinction
10 63 (Wittman *et al.* 2013). Thus, various authors showed a link between the fitness of a species
11
12 64 and its genetic diversity, particularly in mollusks (Mitton & Grant 1984; Garton & Haag 1991;
13
14 65 Zouros 1993; Launey & Hedgecock 2001; Hedgecock *et al.* 2007). ~~Conversely~~In addition, the
15
16 66 reduced genetic variability of an indigenous population could promote the expansion process
17
18 67 of other species that are phylogenetically close. However, very few studies address changes in
19
20 68 ~~the evolution of~~ genetic diversity of an indigenous species under the competitive selection
21
22 69 pressure generated by a biological invader.

23
24 70 The oyster drills *Ocenebra erinacea* (Linnaeus, 1758) and ~~Ocenebrellus~~
25
26 71 ~~inornatus~~Ocenebra inornata (Recluz, 1851) constitute a noteworthy model to study the
27
28 72 genetic effects of indigenous-invasive interactions on indigenous populations. A native of the
29
30 73 northwestern Pacific (Choe & Park 1997; Amano & Vermeij 1998), ~~Ocenebrellus inornatus~~O.
31
32 74 inornata (previously known as Ocenebrellus inornatus; see Houart and Sirenko 2003; Bouchet
33
34 75 and Houart 2014) (Recluz, 1851) has recently invaded European coasts, probably following
35
36 76 massive oyster imports (Pigeot 2000; De Montaudouin & Sauriau 2000; review of Lützen *et*
37
38 77 *al.* 2012). Genetic data suggest that French populations may come from Asia and the United
39
40 78 States (Martel *et al.* 2004a). The introduction of ~~O. inornatus~~O. inornata ~~may~~ has important
41
42 79 economical consequences, as it is a predator of cultivated mollusks (e.g. oysters, blue mussels,
43
44 80 Goulletquer *et al.* 2002). It coexists ~~on~~at several French sites with an indigenous muricid,
45
46 81 ~~O. cenebra~~ *erinacea* (Linnaeus, 1758), which ranges from the straits of Gibraltar to the
47
48 82 Netherlands, and inhabits all British and Mediterranean coasts (Graham 1988). Although *O.*
49
50 83 *erinacea* and ~~O. inornatus~~O. inornata differ in some life history traits (Martel *et al.* 2004c),
51
52 84 ~~they~~these muricid gastropods fill similar ecological niches, and may compete for habitat
53
54 85 (both species live on hard substrates and drill the shell of bivalves to feed on them; e.g.

Lutzen et al 2012). Pigeot *et al.* (2000) recorded a decrease in population density of *O. erinacea*, in parallel to an increase in numbers of *O. inornata* in Marennes-Oléron (Charente-Maritime, France), between 1997 and 1999 (two years after the invasive was first detected). While the introduction and expansion patterns of *O. inornatus* *O. inornata* have been investigated in previous studies (Martel *et al.* 2004a; Martel *et al.* 2004b), its ecological impacts and the influence of on the local native *O. erinacea* species on its capacity of integration are poorly known.

In previous studies (Martel 2003), seven allozyme loci were analyzed on populations of *Ocenebra* *O. erinacea* and *O. inornatus* *Ocenebra inornata* collected in 7 sites of the French Atlantic coast where the two species live in sympatry. These markers revealed genetic diversity indices systematically weaker within the native *O. erinacea* than within the invasive *O. inornatus* *O. inornata*. This result was counterintuitive, as (i) the founder effect linked to an introduction event should lead to a low genetic diversity within the populations of the introduced species, (ii) this phenomenon should be all the more marked as the invasion is recent (review of Sakai *et al.* 2001). Consequently, a lower genetic diversity within the populations of the exogenous species compared to the populations of the indigenous species was expected. It is thus of importance to test whether the genetic diversity of the indigenous species *O. erinacea* is correlated with the presence of the introduced species *O. inornatus* *O. inornata* in zones of sympatry. Indeed, *O. inornatus* *O. inornata* could induce a selective pressure on *O. erinacea* leading to decrease of polymorphism in this local species.

Here, we test this hypothesis by sampling *O. erinacea* from the Atlantic and Mediterranean French coasts, in the presence and absence of *O. inornatus* *Ocenebra inornata*, and by measuring genetic diversity of the native species using the mitochondrial marker *coxI*. While investigating the genetic effects that the presence of *O. inornatus* *O. inornata* may have on sympatric populations of *O. erinacea*, we came across a very strong genetic break between

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

111 Atlantic and Mediterranean populations. This break is detailed and potential biogeographic
112 causes are discussed.

113
114 **Materials and methods**

115
116 Sampling

117 A total of 352 adult specimens of *Ocenebra erinacea* were collected between 1999 and
118 2004 at 15 sites on the French coast, along line transects (<200 m in length). At each site,
119 specimens from different rocks were collected to reduce sampling bias in favor of a particular
120 lineage. The sites were located both within oyster farming zones and unexploited areas (Table
121 1). In order to show a possible impact of the presence of ~~*O. inornatus*~~*Ocenebra inornata* on
122 genetic diversity of *O. erinacea*, five locations where the two species live in sympatry and 10
123 sites free of ~~*O. inornatus*~~*O. inornata* have been sampled. The presence of *O. inornata* was
124 assessed by direct observation. After collection, specimens were stored in 95% ethanol before
125 DNA extraction.

126
127 DNA extraction, amplification and sequencing

128 Total DNA was extracted from <15 mg of foot muscle using Dneasy™ Tissue Kit
129 according to the manufacturer's protocol (Qiagen, Germany). Part of the mitochondrial *coxI*
130 gene was PCR-amplified with the HCO2198/LCO1490 primers (Folmer *et al.* 1994), which
131 are have particularly proved useful for neogastropod studies (e.g. Harasewych *et al.* 1997Zou
132 et al 2011, 2012). Polymorphism at *coxI* is high in ~~*O. inornatus*~~*Ocenebra inornata*, a
133 phylogenetically close species (Martel *et al.* 2004a).

1
2
3
4
5
6
7 134 PCRs were done in 50 μ L, with 1X PCR buffer, 1.85 mM $MgCl_2$, 125 μ M dNTPs,
8
9 135 0.25 μ M of each primer, 1.6 U of Red Hot® DNA Polymerase (ABgene) and about 10 ng of
10
11 136 DNA template. The following cycling profile was performed using a MJResearch PTC 100
12
13 137 Thermal Cycler: initial 5-min denaturation step at 94°C followed by 40 cycles of 30 sec at
14
15 138 94°C, 30 sec at 50°C and 1 min at 72°C, and by a final 5-min extension period at 72°C. PCR
16
17 139 products were purified using MultiScreen-PCR MANU03010 plates (Millipore).

18
19 140 Sequencing was done by GenoScreen (Lille - France) using an ABI PRISM® 3730 XL
20
21 141 Automated DNA Sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA). Sequences
22
23 142 were aligned using ClustalX (Thompson *et al.* 1994).
24

25 143

26 27 144 Data analyses.

28
29 145 Genetic analyses aimed at (i) quantifying and comparing genetic diversity among
30
31 146 populations, (ii) analyzing the spatial distribution of polymorphism and genetic exchanges
32
33 147 among populations, and (iii) studying the evolutionary relationships among populations.
34
35 148 Haplotype number H , number of polymorphic sites S , haplotype diversity H_e and average
36
37 149 per site nucleotide diversity π (Nei 1987) were calculated for each population using the
38
39 150 software DnaSP 5.10.1 (Librado & Rozas 2009).

40
41 151 We tested the null hypothesis of the standard neutral model in Arlequin v.3.5 (Excoffier
42
43 152 & Lischer 2010), by calculating the D and F_s statistics, as defined by Tajima (1989) and Fu
44
45 153 (1997). In each location, we checked the demographic equilibrium of the populations in
46
47 154 Arlequin v.3.5, by calculating the D and F_s statistics, as defined by Tajima (1989) and Fu
48
49 155 (1996). When these statistics are significantly different from zero, populations may have
50
51 156 undergone purifying selection, a selective sweep and/or expansion (<0), or balancing selection
52
53 157 and/or a population decline or overdominant selection and/or a bottleneck (>0). Statistical
54

1
2
3
4
5
6
7 158 significance was tested by generating 10,000 random samples under the hypothesis of
8
9 159 selective neutrality and population equilibrium. These tests were performed for each sampling
10 160 site separately, and also for pooled sites in presence or absence of the invasive.
11
12
13 161 The differentiation index Φ_{ST} (Excoffier *et al.* 1992), an estimator of F_{ST} (Wright 1951)
14
15 162 calculated from frequency values and distances between haplotypes, was computed with
16
17 163 Arlequin v.3.5 (~~Excoffier & Lischer 2010~~). The Kimura 2 Parameter (K2P) model of
18
19 164 nucleotide substitution was used to estimate genetic distances, and 10,000 permutations were
20
21 165 used to test statistical significance under the null hypothesis of no difference between
22
23 166 populations (Excoffier *et al.* 1992).
24
25 167 Finally, a haplotype network was built using the median-joining algorithm
26
27 168 implemented in Network 4.6.1.1 (fluxus-engineering.com, Bandelt 1999). This method is one
28
29 169 of the most accurate for inferring intra-specific networks in the absence of recombination
30
31 170 (Woolley *et al.* 2008).
32
33 171 To help interpret the large genetic divergence observed between Atlantic and
34 172 Mediterranean specimens of *O.~~cenebra~~ erinacea*, we looked for mitochondrial *cox1* data from
35
36 173 the BOLD database (Ratnasingham & Hebert 2007). However, besides three other BOLD
37
38 174 *cox1* sequences from Spanish specimens of *O. erinacea*, we produced the only available
39
40 175 mitochondrial sequences for the genus *Ocenebra*. We therefore used *cox1* sequences from
41
42 176 ~~seven-six~~ species of the closely-related genus *Nucella* Röding, 1798 (e.g. Pascal 2004) to
43
44 177 measure intra-specific and inter-specific genetic distances. *Nucella* and *Ocenebra* are both
45
46 178 characterized by a non-planktonic larval development and lay egg capsules on hard substrates
47
48 179 (Martel *et al.* 2004c; review by Krug 2011). We used the K2P model of nucleotide
49
50 180 substitution (Kimura 1980), ~~a~~-widely used ~~metric~~-in DNA barcoding (Hebert *et al.* 2003;
51
52 181 Barrett & Hebert 2005), to measure genetic distances among *cox1* haplotypes.
53 182

Results

A 550 bp fragment of *cox1* was sequenced for 352 individuals, and 29 haplotypes were identified (GenBank accession numbers AY995771-99; Popset 63109090). Sequences include 37 polymorphic sites, 20 of which are parsimony informative, and one of which has three character states. No indels were observed (Table 2).

Population-level genetic diversity and demographic stability

Genetic diversity is comparable among Atlantic populations of *Ocenebra-erinacea*, but values for the different diversity indices are low compared to other recently-studied marine mollusks. The number of polymorphic sites between two different sequences varies between 0 and 6 among Atlantic populations. Two of these populations (Morbihan and St Quay) are each characterized by a single haplotype, and the 12 other Atlantic sites have no more than five haplotypes (sample sizes provided in Table 1). Consequently, haplotype diversity H_e is low, except for Oléron ($H_e = 0.564$; Table 2). In Fouras, where ~~*Ocenebra inornata*~~ *Ocenebra inornata* and *O. erinacea* are sympatric, the haplotype and nucleotide diversities are respectively four- and six-fold higher in the invasive drill ($H_e = 0.348$ and $\pi \times 10^{-3} = 0.8378$; data from Martel 2003) than in the local one ($H_e = 0.074$ and $\pi \times 10^{-3} = 0.14$).

A single Mediterranean population (Thau) was sampled. The haplotype diversity ($H_e = 0.342$) and the nucleotide diversity ($\pi \times 10^{-3} = 2.52$) are respectively two- and five-fold higher than on the whole of Atlantic populations (Table 2). Moreover, 22 polymorphic sites were found among 37 individuals sampled in Thau, a value considerably higher than the 24 polymorphic sites observed among 315 Atlantic individuals.

1
2
3
4
5
6
7 206 Last, haplotype and nucleotide diversities of *O-cenebra erinacea* populations co-
8
9 207 occurring with *O-inornatusOcenebra inornata* were lower ($H_e = 0.247 \pm 0.0535$ and $\pi \times 10^{-3} =$
10
11 208 0.57 ± 0.65) than for populations located in zones where *O-inornatusO. inornata* was not
12
13 209 detected ($H_e = 0.399 \pm 0.3976$, $\pi \times 10^{-3} = 10.04 \pm 5.52$) (Table 2). However, this pattern is
14
15 210 entirely due to the higher diversity encountered at Thau; when removing this site from the
16
17 211 group of populations that were not found in contact with *O-inornatusO. inornata*, diversity
18
19 212 values dropped significantly ($H_e = 0.179 \pm 0.0368$, $\pi \times 10^{-3} = 0.38 \pm 0.51$). Comparing
20
21 213 molecular diversity at the site level revealed the same pattern (Welch Two Sample t-test,
22
23 214 including Thau; for H_e : $t = -0.83$, $df = 6.22$, $p = 0.44$; for π : $t = -0.16$, $df = 12.81$, $p = 0.88$. In
24
25 215 both cases, results were also non-significant when removing the population of Thau).
26
27 216 Except for one case, the D and F_s statistics were never positive (Table 2). Furthermore,
28
29 217 the only slightly positive D value (deviation from zero non significant) was observed when
30
31 218 sites where the invasive was absent were pooled, including Thau, and this result was therefore
32
33 219 likely influenced by the underlying population structure (see “Genetic differentiation among
34
35 220 populations” Results section). Except for one case (all sites without *O. inornatus* pooled,
36
37 221 deviation from zero non significant), the D and F_s statistics were never positive. There is
38
39 222 therefore no supporting evidence that *O-cenebra erinacea* populations exposed to *O-*
40
41 223 *inornatusO. inornata* suffered from a population declinebottleneck. Some sites populations
42
43 224 exhibited significant negative values of D and F_s , which can be interpreted as signs of
44
45 225 purifying selection, selective sweep and/or population expansion. Particularly, the pooled
46
47 226 Atlantic sites showed significantly negative values for both tests, regardless of whether the
48
49 227 invasive was present or not. These molecular signatures must, however, be interpreted with
50
51 228 care, as they might reflect older demographic events.
52
53 229

Phylogenetic-Genealogical relationships and spatial distribution of haplotypes

Two haplogroups, separated by 18 mutational steps, were observed using the median-joining network (Fig. 1). The first haplogroup (23 haplotypes) was mainly composed of Atlantic specimens, and included one specimen from Thau, characterized by haplotype H26 (separated from other Mediterranean haplotypes by 20 to 22 mutational steps). Haplotypes from this group diverged by ≤ 2 mutations. Haplotype H1 was common (represented in 89% of Atlantic individuals) and central to the Atlantic haplogroup, while the other 22 haplotypes were rare (7 individuals for H3, 3 for H13, 2 for H6, H9, H15, H19 and a single individual for the others) and peripheral to H1. The second haplogroup was strictly composed of Mediterranean individuals. Of six haplotypes from this group, one was common (H21, represented in 82% of Thau individuals), and four were rare (3 individuals for H22 and a single individual for H23-25). Haplotypes from this group diverged by one mutation. The Atlantic samples share no haplotype with the Mediterranean sample (Fig. 2). Except for H1 (present at all sites except Thau), H3 (shared between Loix and Aytré) and H13 (shared between Loix, Aytré and Trébeurden), all haplotypes are private (observed only within one population).

Genetic differentiation among populations

Genetic differentiation among pairs of populations was measured using Φ_{ST} . The population from Thau was significantly differentiated from all other populations. Pairwise Φ_{ST} values ranged between 0.94 and 0.96, corresponding to an important-substantial genetic differentiation between the Mediterranean and Atlantic populations (Table 3). Inside the Atlantic group, no significant differentiation was observed after sequential Bonferroni correction (lowest corrected alpha level for Atlantic populations: 0.00055).

1
2
3
4
5
6
7 254
8
9 255 Levels of intra-specific divergence in *Ocenebra* compared to *Nucella*
10
11 256 Given the differences in haplotype composition and divergence between
12
13 257 Mediterranean and Atlantic sites, we investigated whether the genetic distances correspond to
14
15 258 intra- or inter-specific divergence by comparing *Ocenebra* to its close relative *Nucella*. The
16
17 259 pairwise K2P distance between *Ocenebra* ~~*O. erinacea*~~ haplotypes ranged between 0.18 and
18
19 260 4.54% (maximum observed between haplotypes 20 from Loire and 25 from Thau Lagoon).
20
21 261 Within *Nucella*, K2P was calculated for 532 sequences and 117 haplotypes distributed among
22
23 262 six species, along a 434 bp stretch of *cox1*. Intra-specific distances ranged from 0 to 3.32%,
24
25 263 while inter-specific distance ranged from 4.81 to 12.2%, for specimens distributed over 1000
26
27 264 to 2000 km (East and West coasts of North America, respectively; BOLD database). For
28
29 265 comparison, Zou et al (2011), analyzing 108 neogastropod *cox1* sequences (same gene region
30
31 266 as analysed here; not including *Ocenebra* or *Nucella*) found maximum intra-specific K2P
32
33 267 distances of 2.2% and minimum inter-specific distances of 2.1%.

34 268
35
36
37 269 **Discussion**

38 270
39
40 271 Relation between *Ocenebra* ~~*O. erinacea*~~ and the invasive species ~~*O. inornatus*~~ *Ocenebra*
41
42 272 *inornata*

43
44 273 While a decrease in genetic diversity in response to invaders was reported in the past
45
46 274 (Kim *et al.* 2003), we did not detect such a pattern among *Ocenebra* ~~*O. erinacea*~~ exposed to
47
48 275 the invasive species ~~*O. inornatus*~~ *Ocenebra inornata*. The relatively low polymorphism levels
49
50 276 recorded may have hampered our ability to detect genetic effects of the invasive on the native
51
52 277 species, and the use of ~~more sensitive~~ additional molecular markers such as nuclear
53
54 278 microsatellites or SNPs might further help detect possible demographic events associated with

the presence ~~*O. inornatus*~~*O. inornata*. However, this remains to be tested, as even genome-wide scans can fail at detecting recent demographic events and selective pressures. Riquet *et al.* (2013), for instance, used AFLPs to compare native and invasive populations of the marine mollusk *Crepidula fornicata*. They reported little genetic differentiation among these populations, and detected no F_{ST} outliers out of 344 tested loci. An alternative hypothesis explaining the apparent absence of genetic effects of the invasive on the native is that the competitive and selective pressures inflicted on *O. erinacea* are too ~~mild~~low to have genetic effects (e.g. Wittmann *et al.* 2013). Finally, deviations from neutrality observed for pooled Atlantic sites suggest selection could have shaped the current genetic diversity and could have blurred signatures of demographic processes.

~~*Ocenebra*~~ ~~*O. inornatus*~~ invaded was first documented on the French Atlantic coast in ~~the 1970's~~ the 19750's (De Montaudouin & Sauriau 2000, Pigeot *et al.* 2000), and the specimens of ~~*Ocenebra*~~ *erinacea* used in this study were collected between 1999 and 2004. The introduction of *O. inornata* may have been too recent at the time of sampling for genetic consequences on the native species to be detectable. About ten years later, the distributional landscape of ~~*O. inornatus*~~*O. inornata* on Atlantic coasts have significantly changed, and the invasive is now found as far north as the entrance of the Baltic Sea (Lützen *et al.* 2012). A new survey of the genetic diversity of *O. erinacea* may today unfold the genetic consequences of the invasion by ~~*O. inornatus*~~*O. inornata*, and this study therefore provides a snapshot in time that may help better understand the temporal dynamics of loss of genetic diversity. In addition to sampling in the field, we searched for *Ocenebra* specimens in the collections of the Museum national d'Histoire naturelle in Paris (France), in order to look for genetic diversity in *O. erinacea* specimens collected prior to, or soon after the invasion by *O. inornata* ~~*Ocenebrellus*~~ (MNHN voucher numbers IM-2008-7101, IM-2008-7102, IM-2008-7103). Unfortunately, we were not able to amplify the *coxI* marker from these specimens.

Remarkably low genetic diversity of *Ocenebra*-*erinacea* populations

Mitochondrial genetic diversity, as measured using part of *cox1*, was low relative to what was observed in other marine mollusks. Overall, *Ocenebra*-*erinacea* haplotype and nucleotide diversities were (disregarding the sample from Thau, see below) $H_e = 0.18-0.25$ and $\pi \times 10^{-3} = 0.38-0.57$ (Table 2). Comparatively, $H_e = 0.684$ and $\pi \times 10^{-3} = 2.25$ for *O. inornatus* *Ocenebra inornata* in its natural range (data from Martel *et al.* 2004a), $H_e = 0.734$ and $\pi \times 10^{-3} = 14.78$ in the gastropod *Cyclope neritea* (Simon-Bouhet *et al.* 2006), and $H_e = 0.720$ and $\pi \times 10^{-3} = 89.84$ in the bivalve *Macoma balthica* (Becquet *et al.* 2012).

The low genetic diversity observed at *cox1* was consistent with the low diversity observed using allozymes: Martel (2003) reported that the number of alleles N_{all} and the observed heterozygosity H_o characterizing the Atlantic populations of *Ocenebra* *erinacea* are respectively two to four times, and twenty to thirty times lower ($N_{all} = 1.1 \pm 0.1$; $H_o = 0.01 \pm 0.01$; mean $\pm$ SD) than in other marine gastropods sampled in their native range, such as *Bedevea hanleyi* ($N_{all} = 2.2 \pm 0.1$, $H_o = 0.30 \pm 0.02$; Hoskin 2000), *Drupella* *sp.* ($N_{all} = 2.3 \pm 1.0$, $H_o = 0.25$; Johnson & Cumming 1995), and *Littorina striata* ($N_{all} = 4.2 \pm 1.0$, $H_o = 0.18 \pm 0.17$; De Wolf *et al.* 2000). Congruent patterns across mitochondrial and allozyme markers thus suggest a low genetic diversity in these populations rather than an absence of variability at *cox1*. Still, our sampling remains restricted compared to the native range of the species, and additional monitoring may reveal new patterns of genetic diversity.

Genetic diversity and connectivity among populations

The genetic diversity of the Mediterranean population was among the highest ~~str than that of each Atlantic population (except Oléron,~~ Table 2). The oyster farmers of the Thau

lagoon, one of the main shell farming areas of the French Mediterranean coasts, carry out commercial exchanges with distant production sites, and *Ocenebra erinacea* is likely to be transferred during these exchanges. In fact, the morphological survey done by Berrou *et al.* (2004) evidenced exchanges ~~between from~~ Oléron Island ~~and to~~ Thau Lagoon (this is congruent with the observation of the Atlantic haplotype H26 being observed at Thau; Figs. 1-2). In our study, the high genetic diversity observed in Thau Lagoon could be the result of the introduction of Mediterranean specimens imported from other production sites such as Oléron. As no Mediterranean site was sampled other than Thau Lagoon, the artificial mixing induced by shellfish exchanges cannot be further evaluated here.

Alternatively, the difference in genetic diversity observed between the Atlantic and the Mediterranean population of Thau lagoon could be explained by historical and biogeographical factors, and/or selection. *Ocenebra erinacea* is not well ~~adapted~~ to cold water and Belgium currently constitutes the northern limit of its natural range (Graham 1988). Consequently, the species may have found, as other marine species (e.g. Nikula & Väinölä 2003, Ladhar-Chaabouni *et al.* 2010), a refugium on the Iberian coast or in the Mediterranean basin during past glaciations. *O. erinacea* may have disappeared from the French Atlantic coasts during the Würm Glacial period (115,000 to 10,000 years BP) but survived on the coasts of the Iberian peninsula, which is known as one of the major Pleistocene refugia (Gómez & Lunz 2007). At the end of this climatic crisis, a reduced number of individuals from southern refuges may have reached northern coasts. Maggs *et al.* (2008), reviewing molecular signatures of glacial refugia on marine species, made predictions of low genetic and haplotype diversity in northern regions previously covered by ice sheets, and comparatively high diversity in refugial southern regions (and see Hewitt 1996). These predictions are generally met for *O. erinacea*: a mitochondrial genealogy matching geography, but additional sampling from the Iberian peninsula and the Mediterranean Sea would be necessary to further

1
2
3
4
5
6 353 | characterize the historical biogeography of this species. Given *O. erinacea*'s maladaptation to
7
8 354 | cold-water, another possibility is that selection (either purifying selection, or selective sweeps)
9
10 355 | linked to differences in water temperature between Thau and the Atlantic sites produced the
11
12 356 | observed patterns of genetic diversity. The negative Tajima's *D* and Fu's *F_s* observed for the
13
14 357 | Atlantic population (in absence and presence of the invasive) would support this scenario, and
15
16 358 | Hewitt's "northern purity, southern richness" genetic diversity pattern (Magg's et al. Model
17
18 359 | Ha).

19
20 360 | One potential consequence of biogeographic divergence between Atlantic and
21
22 361 | Mediterranean populations is the emergence of new species (Hewitt 1996, 2004). Recently,
23
24 362 | Salicini *et al.* (2013) have shown that in the bat *Myotis naterreri*, a complex of four cryptic
25
26 363 | species exist in the Western Palearctic region (central and southern Europe, northwestern
27
28 364 | Maghreb), each species coinciding with a glacial refugium. In *Ocenebra- erinacea*, inter-clade
29
30 365 | divergence overlaps with the inter-specific distances observed in the closely-related genus
31
32 366 | *Nucella* (even though the geographical distances separating *Nucella* specimens were greater
33
34 367 | than the distances separating *O. erinacea* specimens; see Bergsten *et al.* 2012). In addition, *O.*
35
36 368 | *erinacea* specimens from the Atlantic and Mediterranean can readily be distinguished using
37
38 369 | morphology, and the morphological distance between Atlantic and Mediterranean *O. erinacea*
39
40 370 | is comparable to what is observed between *O. erinacea* and *Ocenebra- brevirobusta* Houart
41
42 371 | 2000 (Berrou *et al.* 2004). It is therefore possible that the Atlantic and Mediterranean clades
43
44 372 | sampled for this study belong to groups in incipient stages of speciation, or even undescribed
45
46 373 | species.
47
48 374

Acknowledgements

We thank Stéphane Robert (IFREMER, La Tremblade, France), Yvonne Pascal, Michel Pascal (INRA Rennes, France), François Siorat (LPO), Armel Deniau (LPO), Marie-Gabrielle Vitre, Paul Wendling, Françoise Dreau, Christian Bussière, Isabelle Kuhne, Jean-Paul Egreteau, José Simone Guézou, Sandrine Sourisseau, Xavier de Montaudouin (University of Bordeaux, France), Pierre-Guy Sauriau (IFREMER, L'Houmeau, France) for their help with field sampling, Jacques Pigeot for his insights and help with identifications, Stéphane Robert (IFREMER, La Tremblade, France), Philippe Goulletquer (IFREMER, La Tremblade, France) and Yves Pichot (IFREMER, Sète, France) for information on shellfish transfers in France, Pierre Lozouet at the Muséum national d'Histoire naturelle (Paris, France) for granting access to the *Ocenebra* collection, and Alice Saunier for her help in the lab. This work was funded by the Conseil Général de Charente-Maritime and the Ministère de l'Ecologie et du Développement Durable (Program INVABIO no. D4E/SRP/01115). Salaries for EP & AV were covered by a grant to the Poitou-Charentes region (Contrat de Projet État-Région 2007-2013), by a grant from the Fond Européen de Développement Régional (EP), and by Actions Internationales et Rayonnement of the University of La Rochelle (AV). We thank the three anonymous reviewers and the editor, whose comments significantly improved the manuscript. This work is dedicated to the memory of Michel Pascal.

References

- Amano, K., G.J. Vermeij (1998) Taxonomy and evolution of the genus *Ocinebrellus* (Gastropoda: Muricidae) in Japan. *Paleontological Research*, **2**, 199-212.
- Bandelt, H., P. Forster, et al. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37-48.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Barrett, R., P.D.N. Hebert (2005) Identifying spiders through DNA barcodes. *Canadian Journal of Zoology*, **83**, 481–491.

Becquet, V., B. Simon-Bouhet, et al. (2012) Glacial refugium versus range limit: conservation genetics of *Macoma balthica*, a key species in the Bay of Biscay (France). *Journal of Experimental Marine Biology and Ecology*, **432-433**, 73-82.

Bergsten, J., D.T. Bilton, T. Fujisawa, et al. (2012) The effect of geographical scale of sampling on DNA barcoding. *Systematic Biology*, **61**, 851–869.

Berrou, V., D. Merle, et al. (2004) Comparative morphology of Pliocene, Quaternary and Recent shells of *Ocenebra erinaceus* (Linnaeus, 1758) and *O. brevirobusta* Houart, 2000 (Mollusca, Muricidae, Ocenebrinae): reflections on the intra- and interspecific variations. *Geodiversitas*, **26**(2), 263-295.

Bouchet, P., R. Houart (2014). *Ocenebra inornata* (Récluz, 1851). Accessed through: World Register of Marine Species at <http://www.marinespecies.org/aphia.php?p=taxdetails&id=403745> on 2014-05-02.

Carlton, J.T. (1989) Man’s role in changing the face of the ocean: biological invasions and implications for conservation of near shore environments. *Conservation Biology* **3**: 265-273.

Carlton, J.T. (1992) Introduced marine and estuarine mollusks of North America: an end-of-the-20th-century perspective. *Journal of Shellfish Research*, **11**, 489-505.

Choe, B.L., J.K. Park (1997) Description of Muricid species (Gastropoda: Neogastropoda) collected from the coastal areas of south Korea. *Journal of Biological Science*, **1**, 281-296.

De Montaudouin, X., P.-G. Sauriau (2000) Contribution to a synopsis of marine species richness in the Pertuis Charentais Sea with new insights in soft-bottom macrofauna of the Marennes-Oléron Bay. *Cahiers de Biologie Marine*, **41**, 188-222.

- 424 De Wolf, H., R. Verhagen, et al. (2000) Large scale population structure and gene flow in the
425 planktonic developing periwinkle *Littorina striata* (Mollusca: Gastropoda), in
426 Macronésia. *Journal of Experimental Marine Biology and Ecology*, **246**, 69-83.
- 427 Elton, C. (1958). *The ecology of invasions by animals and plants*. University of Chicago
428 Press.
- 429 Excoffier, L., H.E.L. Lischer (2010) Arlequin suite ver 3.5: A new series of programs to
430 perform population genetics analyses under Linux and Windows. *Molecular Ecology*
431 *Resources*, **10**, 564-567.
- 432 Excoffier, L., P.E. Smouse, et al. (1992) Analysis of Molecular Variance Inferred From
433 Metric Distances Among DNA Haplotypes: Application to Human Mitochondrial DNA
434 Restriction Data. *Genetics*, **131**, 479-491.
- 435 Folmer, O., M. Black, et al. (1994) DNA primers for amplification of mitochondrial
436 cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine*
437 *Biology and Biotechnology*, **3**, 294-299.
- 438 Frankham, R. (1995) Conservation genetics. *Annual Review of Genetics*, **29**, 305-327.
- 439 Fu, Y.-X. (1997) Statistical tests of neutrality of mutations against population growth,
440 hitchhiking and background selection. *Genetics*, **147**, 915-925.
- 441 ~~Fu, Y.-X. (1996) New Statistical Tests of Neutrality for DNA Samples From a Population.~~
442 ~~*Genetics*, **143**, 557-570.~~
- 443 Garton, D.W., W.R. Haag (1991) Heterozygosity, shell length and metabolism in the
444 European mussel, *Dreissena polymorpha*, from a recently established population in lake
445 Erie. *Comparative Biochemistry and Physiology*, **99**, 45-48.
- 446 Goulletquer, P., G. Bachelet, et al. (2002) Open Atlantic coast of Europe - a century of
447 introduced species into French waters. In: E. Leppäkoski, S. Gollash & S. Olenin (Eds).

1
2
3
4
5
6 448 *Invasive species of Europe - Distribution, impact and management*. Kluwer Academic
7
8 449 Publishers, Dordrecht / Boston / London.
9
10 450 Gómez, A., D.H. Lunt (2007) Refugia within Refugia: Patterns of Phylogeographic
11
12 451 Concordance in the Iberian Peninsula. In: S. Weiss, N. Ferrand (Eds). *Phylogeography of*
13
14 452 *Southern European Refugia*. Netherlands: Springer, volume III, pp. 155-188.
15
16 453 Graham, A. (1998). *Molluscs: Prosobranchs and Pyramidellid Gastropods: Keys and Notes*
17
18 454 *for the Identification of the Species*. Linnean Society of London.
19
20 455 ~~Harasewych, M.G., S.L. Adamkewicz, et al. (1997) Neogastropod phylogeny: a molecular~~
21
22 456 ~~perspective. *Journal of Molluscan Studies*, **63**, 327-351.~~
23
24
25 457 Hebert, P.D.N., A. Cywinska, et al. (2003) Biological identifications through DNA barcodes.
26
27 458 *Proceedings of the Royal Society of London Series B-Biological Sciences*, **270**, 313–321.
28
29 459 ~~Hedgecock, D., L. Jing-Zhong, S. DeCola, C.D. Haudenschield, E. Meyer, D.T. Manahan, B.~~
30
31 460 ~~Bowen (2007) Transcriptomic analysis of growth heterosis in larval Pacific oysters~~
32
33 461 ~~(*Crassostrea gigas*). *Proceedings of the National Academy of Sciences of the United States*~~
34
35 462 ~~*of America*, **104**, 2313-2318.~~
36
37 463 Hewitt, G.W. (1996) Some genetic consequences of ice ages, and their role in divergence and
38
39 464 speciation. *Biological Journal of the Linnean Society*, **58**, 247-276.
40
41 465 Hewitt, G.M. (2004) Genetic consequences of climatic oscillations in the quaternary.
42
43 466 *Philosophical transactions of the Royal Society of London. Series B, Biological Sciences*,
44
45 467 **359**, 183–195.
46
47 468 Hoskin, M.G. (2000) Effects of the east Australian current on the genetic structure of a direct
48
49 469 developing muricid snail (*Bedeva hanleyi*, Angas): variability within and among local
50
51 470 populations. *Biological Journal of the Linnean Society*, **69**, 245-262.
52
53
54
55
56
57
58
59
60

- Houart, R., B.I. Sirenko (2003) Review of the Recent species of *Ocenebra* Gray, 1847 and *Ocinebrellus* Jousseaume, 1880 in the Northwestern Pacific. *Ruthenica*, **13**, 53-74.
- Johnson, M.S., R.L. Cumming (1995) Genetic distinctness of three widespread and morphologically variable species of *Drupella* (Gastropoda: Muricidae). *Coral Reefs*, **14**, 71-78.
- Kim, M.S., S.D. Brunsfeld, et al. (2003) Effect of white pine blister rust (*Cronartium ribicola*) and rust-resistance breeding on genetic variation in western white pine (*Pinus monticola*). *Theoretical and Applied Genetics*, **106**, 1004-1010.
- Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide-sequences. *Journal of Molecular Evolution*, **16**, 111-120.
- Krug, P. (2011) Patterns of speciation in marine gastropods: a review of the phylogenetic evidence for localized radiations in the sea. *American Malacological Bulletin*, **29**, 169-186.
- Ladhar-Chaabouni, R., A. Hamza-Chaffai, et al. (2010) A pilot study of genetic differentiation between two phenotypes of a Mediterranean population of the bivalve *Cerastoderma glaucum* and genetic discrimination with other *Cerastoderma glaucum* and *Cerastoderma edule* populations outside the Mediterranean. *Marine Ecology*, **31**, 355-363.
- Lande, R. (1995) Mutation and conservation. *Conservation Biology*, **9**, 782-791.
- Launey, S., D. Hedgecock (2001) High genetic load in the Pacific oyster *Crassostrea gigas*. *Genetics*, **159**, 255-265.
- Librado, P., J. Rozas (2009) DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451-1452.
- Lockwood, J.L., M.F. Hoopes, M.P. Marchetti (2007) *Invasion Ecology*. Blackwell Publishing, Oxford, UK. 304 pp.

1
2
3
4
5
6 496 Lützen, J., M. Faasse, et al. (2012) The Japanese oyster drill *Ocenebrellus inornatus* (Récluz,
7
8 497 1851) (Mollusca, Gastropoda, Muricidae), introduced to the Limfjord, Denmark.
9
10 498 *Aquatic Invasions*, **7**(2), 181-191.
11
12 499 Mack, R.N., D. Simberloff, et al. (2000) Biotic invasions: causes, epidemiology, global
13
14 500 consequences and control. *Ecological Application*, **10**, 690-710.
15
16 501 Maggs, C.A., R. Castilho, et al. (2008) Evaluating signatures of glacial refugia for North
17
18 502 Atlantic benthic marine taxa. *Ecology*, **89**, S108-S122.
19
20 503 Martel C. (2003) *Invasions biologiques et perturbations anthropiques des écosystèmes*
21
22 504 *littoraux : source, profil d'expansion et impacts d'un gastéropode marin*, *Ocenebrellus*
23
24 505 *inornatus*, *introduit accidentellement sur les côtes atlantiques françaises*. Doctoral thesis,
25
26 506 Université de La Rochelle. 122 pp & Annexes.
27
28 507 Martel, C., D. Bourguet, et al. (2004b) Invasion by the marine gastropod *Ocenebrellus*
29
30 508 *inornatus* in France. II. Expansion along the Atlantic coast. *Marine Ecology Progress*
31
32 509 *Series*, **273**, 163-172.
33
34 510 Martel, C., J.M. Guarini, et al. (2004c) Invasion by the marine gastropod *Ocenebrellus*
35
36 511 *inornatus* in France. III. Comparison of biological traits with the resident species
37
38 512 *Ocenebra erinacea*. *Marine Biology*, **146**, 93-102.
39
40 513 Martel, C., F. Viard, et al. (2004a) Invasion by the marine gastropod *Ocenebrellus inornatus*
41
42 514 in France. I. Scenario for the source of introduction. *Journal of Experimental Marine*
43
44 515 *Biology and Ecology*, **305**, 155-170.
45
46 516 Mitton, J.B., M.C. Grant (1984) Associations among protein heterozygosity, growth rate and
47
48 517 developmental homeostasis. *Annual review of Ecology and Systematics*, **15**, 479-499.
49
50 518 Mooney, H.A., E.E. Cleland (2001) The evolutionary impact of invasive species. *Proceedings*
51
52 519 *of the National Academy of Sciences of the United States of America*, **98**, 5446-5451.
53
54 520 Nei M. (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

- 521 Nikula, R., R. Väinölä (2003) Phylogeography of *Cerastoderma glaucum* (Bivalvia:
522 Cardiidae) across Europe: a major break in the Eastern Mediterranean. *Marine Biology*,
523 **143**, 339-350.
- 524 Pante, E., B. Simon-Bouhet (2013) marmap: A Package for Importing, Plotting and Analyzing
525 Bathymetric and Topographic Data in R. *PloS One*, **8**(9), e73051.
- 526 Parmesan C., G. Yohe (2003) A globally coherent fingerprint of climate change impacts
527 across natural systems. *Nature*, **421**, 37-42.
- 528 Pascal P.-Y. (2004) *Rôle des espèces autochtones dans les processus d'invasions biologiques*
529 *marines: l'exemple du perceur invasif Ocinebrellus inornatus et du perceur autochtone*
530 *Ocenebra erinacea sur les côtes françaises*. Masters Thesis, Université de La Rochelle,
531 47pp.
- 532 Pigeot, J., P. Miramand, et al. (2000) Présence d'un nouveau prédateur de l'huître creuse,
533 *Ocinebrellus inornatus* (Recluz, 1851), dans le bassin conchylicole de Marennes-
534 Oléron. *Comptes-rendus de l'Académie des Sciences de Paris*, **323**, 697-703.
- 535 Ratnasingham, S., P.D.N. Hebert (2007) BOLD : The Barcode of Life Data System BOLD:
536 The Barcode of Life Data System (<http://www.barcodinglife.org>). *Molecular Ecology*
537 *Notes*, **7**, 355-364.
- 538 Riquet, F., C. Daguin-Thiébaud, et al. (2013) Contrasting patterns of genome-wide
539 polymorphism in the native and invasive range of the marine mollusc *Crepidula*
540 *forficata*. *Molecular Ecology*, **7**, 355-364.
- 541 Ruiz, G.M., T.K. Rawlings, et al. (2000) Global spread of microorganisms by ships: ballast
542 water discharged from vessels harbours a cocktail of potential pathogens. *Nature*, **408**,
543 49-50.
- 544 Sakai, A.K., F.W. Allendorf, et al. (2001) The population biology of invasive species. *Annual*
545 *Review of Ecology and Systematics*, **32**, 305-332.

1
2
3
4
5
6 546 Salicini I., C. Ibañez , et al. (2013) Deep differentiation between and within Mediterranean
7
8 547 glacial refugia in a flying mammal, the *Myotis nattereri* bat complex. *Journal of*
9
10 548 *Biogeography*, **40**, 1182–1193.
11
12 549 Seebens H., M.T. Gastner, et al. (2013) The risk of marine bioinvasion caused by global
13
14 550 shipping. *Ecology Letters*, **6**, 782-790.
15
16 551 Simon-Bouhet, B., P. Garcia-Meunier, et al. (2006) Multiple introductions promote range
17
18 552 expansion of the mollusc *Cyclope neritea* (Nassariidae) in France: evidence from
19
20 553 mitochondrial sequence data. *Molecular Ecology*, **15**, 1699-1711.
21
22 554 Strauss, S.Y., J.A. Lau, et al. (2006) Evolutionary responses of natives to introduced species:
23
24 555 what do introductions tell us about natural communities? *Ecology Letters*, **9**, 357-374.
25
26 556 Tajima, F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA
27
28 557 polymorphism. *Genetics*, **123**(3), 585-595.
29
30 558 Thompson, J.D., T.J. Gibson, et al. (1997) The ClustalX windows interface: flexible strategies
31
32 559 for multiple sequence alignment aided by quality analysis tools. *Nucleic Acid Research*,
33
34 560 **24**, 4876-4882.
35
36 561 Wittman, M.J., M. Hutzenthaler, et al. (2013) Ecological and genetic effects of introduced
37
38 562 species on their native competitors. *Theoretical Population Biology*, **84**, 25-35.
39
40 563 Woolley, S.M., D. Posada, et al. (2008) A comparison of phylogenetic network methods using
41
42 564 computer simulation. *PloS One*, **3**, e1913.
43
44 565 Wright, S. (1951) The genetical structure of populations. *Annals of Human Genetics*, **15**, 323-
45
46 566 354.
47 567 Zou, S., Q. Li, L. Kong, H. Yu, X. Zheng (2011) Comparing the Usefulness of Distance,
48
49 568 Monophyly and Character-Based DNA Barcoding Methods in Species Identification: A
50
51 569 Case Study of Neogastropoda. PLoS One, 6, e26619.
52
53
54
55
56
57
58
59
60

- 570 [Zou, S., Q. Li, L. Kong \(2012\) Multigene barcoding and phylogeny of geographically](#)
571 [widespread muricids \(Gastropoda: Neogastropoda\) along the coast of china. *Marine*](#)
572 [Biotechnology](#), **14**, 21–34.
- 573 Zouros, E. (1993) Associative overdominance evaluating the effects of inbreeding and linkage
574 disequilibrium. *Genetica*, **89**, 35–46.

Table 1. ~~Sampling sites~~*Ocenebra erinacea* ~~Collection-collection~~ sites (listed from North to South, following the coastline), site name abbreviations (Ab., as used in Figure 2), number of *O. erinacea* specimens collected and sequenced (n), geographical coordinates, ~~year of collection~~, and characteristics of the locations: presence (+) or absence (-) of oyster farms and of ~~*O. inornatus*~~*O. inornata*.

Location	Ab.	n	Latitude	Longitude	Shellfish area	<i>O. inornatus</i>
Blainville	Bl	31	49°03' N	1°36' W	+	-
Chaussey	Ch	25	48°52' N	1°48' W	+	-
Saint Malo	SM	22	48°39' N	2°01' W	-	-
Saint Quay	SQ	23	48°39' N	2°50' W	-	-
Trébeurden	Tr	24	48°48' N	3°35' W	-	-
Crozon	Cr	23	48°17' N	4°27' W	-	-
Le Croisic	LC	22	47°18' N	2°31' W	-	-
Morbihan	Mo	12	47°33' N	2°51' W	-	-
Bourgneuf	Bo	24	47°01' N	2°01' W	+	+
Loix	Lo	28	46°13' N	1°24' W	+	+
Aytré	Ay	20	46°06' N	1°07' W	+	+
Fouras	Fo	27	46°00' N	1°07' W	+	+
Oléron	Ol	13	45°53' N	1°10' W	+	+
Arcahe	Ar	21	44°40' N	1°12' W	+	-
Thau	Th	37	43°24' N	3°35' W	+	-

Location	Ab.	n	Latitude	Longitude	Year of collection	Shellfish area	Presence of <i>O. inornata</i>
Blainville	Bl	31	49°03' N	1°36' W	2003	+	-
Chaussey	Ch	25	48°52' N	1°48' W	2003	+	-
Saint Malo	SM	22	48°39' N	2°01' W	2004	-	-
Saint Quay	SQ	23	48°39' N	2°50' W	2004	-	-
Trébeurden	Tr	24	48°48' N	3°35' W	2004	-	-
Crozon	Cr	23	48°17' N	4°27' W	2004	-	-
Le Croisic	LC	22	47°18' N	2°31' W	2004	-	-
Morbihan	Mo	12	47°33' N	2°51' W	2004	-	-

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

582
583

Bourgneuf	Bo	24	47°01' N	2°01' W	2003	±	±
Loix	Lo	28	46°13' N	1°24' W	2003	±	±
Aytré	Ay	20	46°06' N	1°07' W	2003	±	±
Fouras	Fo	27	46°00' N	1°07' W	2004	±	±
Oléron	Ol	13	45°53' N	1°10' W	2004	±	±
Arcachon	Ar	21	44°40' N	1°12' W	1999	±	±
Thau	Th	37	43°24' N	3°35' W	1999	±	±

For Peer Review

1
2
3
4
5
6
7 584 Table 2. Molecular diversity of populations and results of the neutrality tests. The number of
8
9 585 segregating sites (*S*), the number of haplotypes (*H*), the haplotype diversity ($H_e \pm$ one standard
10 586 deviation), and the nucleotide diversity ($\pi \pm$ one standard deviation) are given for each
11
12 587 sampling site, site groups in presence and in absence of the invasive (with and without the
13
14 588 Mediterranean population of Thau), and for the entire data set. Sites where *O.*
15
16 589 *inornatus**Ocenebra inornata* was present are indicated by an asterisk. For neutrality tests of
17
18 590 Tajima and Fu, statistical significance after sequential Bonferroni correction is indicated with
19
20 591 an asterisk.
21
22 592
23
24 593

Sampling-sites	<i>S</i>	<i>H</i>	$H_e \pm SD$	$\pi \pm SD (x10^{-3})$	Tajima's <i>D</i>	Fu's <i>F_s</i>
Blainville	1	2	0.065 ± 0.059	0.12 ± 0.28	-1.14	-1.24*
Chaussey	3	4	0.230 ± 0.110	0.44 ± 0.58	-1.73	-3.08*
Saint Malo	1	2	0.091 ± 0.081	0.17 ± 0.34	-1.16	-0.96
Saint Quay	0	1	0	0	0.00	0.00
Trébeurden	5	5	0.377 ± 0.122	0.90 ± 0.89	-1.83	-2.80*
Crozon	2	3	0.316 ± 0.118	0.60 ± 0.70	-0.86	-0.87
Le-Croisie	4	4	0.333 ± 0.124	0.81 ± 0.84	-1.67	-1.74
Morbihan	0	1	0	0	0.00	0.00
Bourgneuf*	2	2	0.083 ± 0.075	0.30 ± 0.47	-1.51	-0.19
Loix*	5	5	0.270 ± 0.109	0.65 ± 0.72	-2.01*	-3.57*
Aytré*	4	4	0.363 ± 0.131	0.89 ± 0.89	-1.64	-1.61
Fouras*	1	2	0.074 ± 0.067	0.14 ± 0.30	-1.15	-1.12
Oléron*	2	3	0.564 ± 0.112	1.12 ± 1.06	-0.13	-0.17
Arcaehon	1	2	0.095 ± 0.084	0.17 ± 0.35	-1.16	-0.92
Thau	22	6	0.342 ± 0.098	2.52 ± 1.76	-2.50*	-0.42
Sites in-presence of invasive	11	9	0.247 ± 0.0535	0.57 ± 0.65	-2.10*	-9.42*
Sites in-absence of invasive	31	22	0.399 ± 0.3976	10.04 ± 5.52	0.22	-0.06
Sites in-absence of invasive (without Thau)	16	16	0.179 ± 0.0368	0.38 ± 0.51	-2.38*	<-10*
All-populations	37	29	0.356 ± 0.0325	7.63 ± 4.20	-0.81	-4.97
Sampling sites	<i>S</i>	<i>H</i>	$H_e \pm SD$	$\pi \pm SD (x10^{-3})$	Tajima's <i>D</i>	Fu's <i>F_s</i>
Blainville	1	2	0.065 ± 0.059	0.12 ± 0.28	-1.14	-1.24*
Chaussey	3	4	0.230 ± 0.110	0.44 ± 0.58	-1.73	-3.08*
Saint Malo	1	2	0.091 ± 0.081	0.17 ± 0.34	-1.16	-0.96

<u>Saint Quay</u>	<u>0</u>	<u>1</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
<u>Trébeurden</u>	<u>5</u>	<u>5</u>	<u>0.377 ± 0.122</u>	<u>0.90 ± 0.89</u>	<u>-1.83</u>	<u>-2.80*</u>
<u>Crozon</u>	<u>2</u>	<u>3</u>	<u>0.316 ± 0.118</u>	<u>0.60 ± 0.70</u>	<u>-0.86</u>	<u>-0.87</u>
<u>Le Croisic</u>	<u>4</u>	<u>4</u>	<u>0.333 ± 0.124</u>	<u>0.81 ± 0.84</u>	<u>-1.67</u>	<u>-1.74</u>
<u>Morbihan</u>	<u>0</u>	<u>1</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
<u>Bourgneuf *</u>	<u>2</u>	<u>2</u>	<u>0.083 ± 0.075</u>	<u>0.30 ± 0.47</u>	<u>-1.51</u>	<u>-0.19</u>
<u>Loix *</u>	<u>5</u>	<u>5</u>	<u>0.270 ± 0.109</u>	<u>0.65 ± 0.72</u>	<u>-2.01*</u>	<u>-3.57*</u>
<u>Aytré *</u>	<u>4</u>	<u>4</u>	<u>0.363 ± 0.131</u>	<u>0.89 ± 0.89</u>	<u>-1.64</u>	<u>-1.61</u>
<u>Fouras *</u>	<u>1</u>	<u>2</u>	<u>0.074 ± 0.067</u>	<u>0.14 ± 0.30</u>	<u>-1.15</u>	<u>-1.12</u>
<u>Oléron *</u>	<u>2</u>	<u>3</u>	<u>0.564 ± 0.112</u>	<u>1.12 ± 1.06</u>	<u>-0.13</u>	<u>-0.17</u>
<u>Arcachon</u>	<u>1</u>	<u>2</u>	<u>0.095 ± 0.084</u>	<u>0.17 ± 0.35</u>	<u>-1.16</u>	<u>-0.92</u>
<u>Thau</u>	<u>22</u>	<u>6</u>	<u>0.342 ± 0.098</u>	<u>2.52 ± 1.76</u>	<u>-2.50*</u>	<u>-0.42</u>
<u>Sites in presence of invasive</u>	<u>11</u>	<u>9</u>	<u>0.247 ± 0.0535</u>	<u>0.57 ± 0.65</u>	<u>-2.10*</u>	<u>-9.42*</u>
<u>Sites in absence of invasive</u>	<u>31</u>	<u>22</u>	<u>0.399 ± 0.3976</u>	<u>10.04 ± 5.52</u>	<u>0.22</u>	<u>-0.06</u>
<u>Sites in absence of invasive (without Thau)</u>	<u>16</u>	<u>16</u>	<u>0.179 ± 0.0368</u>	<u>0.38 ± 0.51</u>	<u>-2.38*</u>	<u>< -10*</u>
<u>All populations</u>	<u>37</u>	<u>29</u>	<u>0.356 ± 0.0325</u>	<u>7.63 ± 4.20</u>	<u>-0.81</u>	<u>-4.97</u>

Table 3. Pairwise Φ_{ST} values calculated using the Kimura 2 Parameter model of nucleotide substitution. Only pairwise comparisons involving the population of Thau are statistically significant after sequential Bonferroni correction ($p=0$).

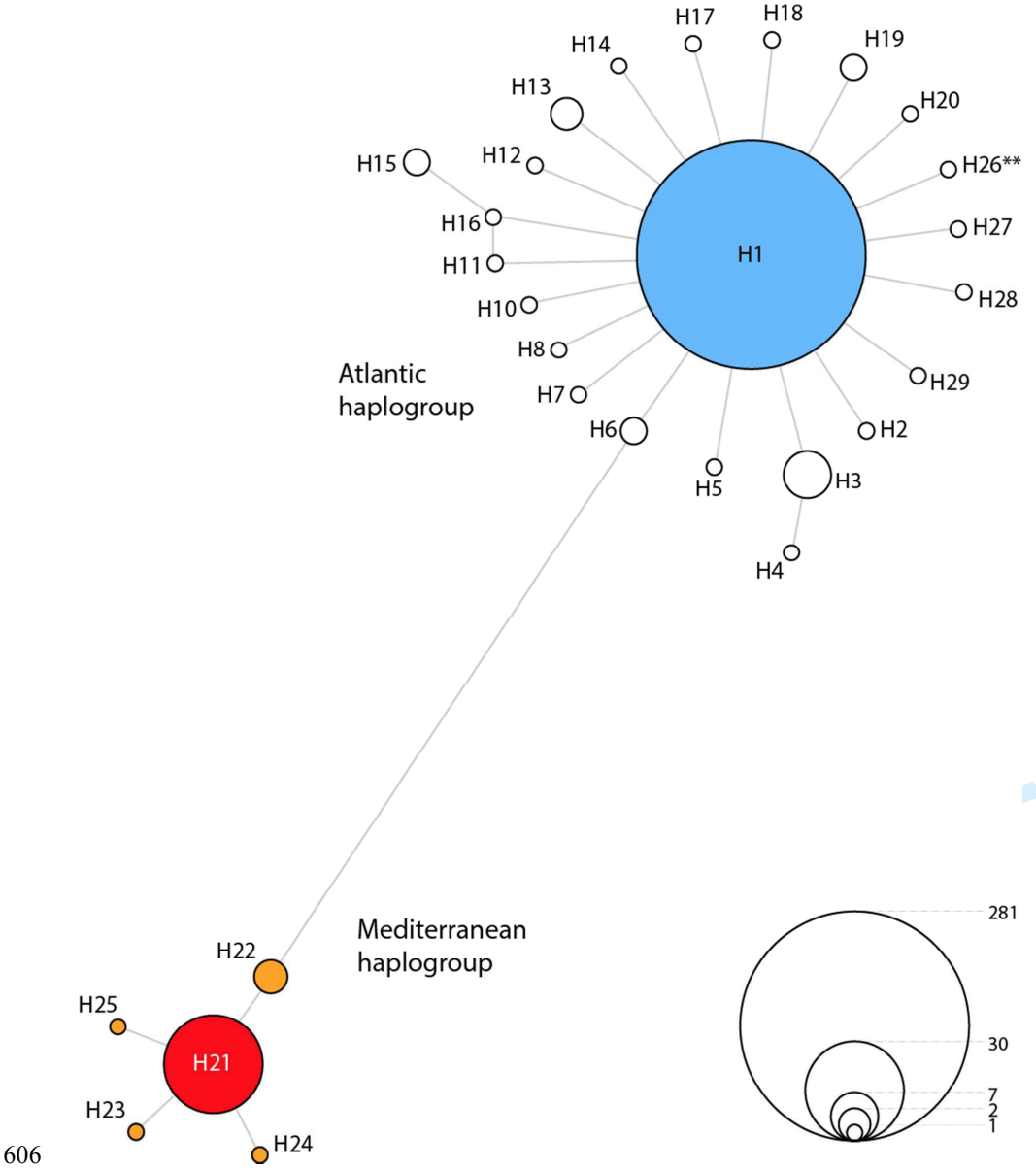
598

599

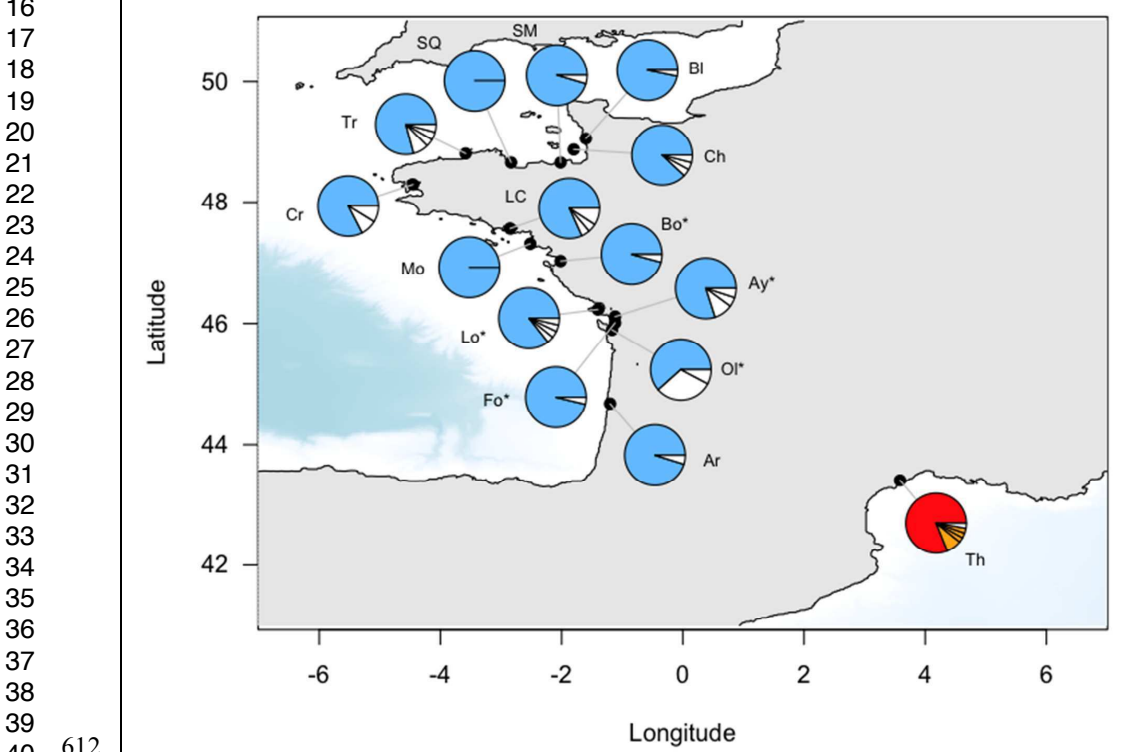
600

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Blainville	0														
2. Chausey	0.0048	0													
3. Saint Malo	0.00242	0.00244	0												
4. Saint Quay	0.01001	0.00356	0.00207	0											
5. Trebeurden	0.02194	0.01045	0.00921	0.01207	0										
6. Crozon	0.05037	0.02772	0.03452	0.04529	0.02682	0									
7. Le Croisic	0.03001	0.01442	0.01577	0.02118	0.01654	0.01135	0								
8. Morbihan	0.03708	0.03371	0.03067	0	0.02156	0.00476	0.01543	0							
9. Bourgneuf	0.00458	0.00021	0.00102	0.00181	0.01083	0.0316	0.01598	0.03274	0						
10. Loix	0.00242	-0.0008	0.00559	0.00747	0.00258	0.02217	0.012	-0.0358	0.01337	0					
11. Aytré	0.03811	0.01838	0.02156	0.0293	0.00956	0.03281	0.02045	0.01049	0.00555	0.01096	0				
12. Oleron	0.28743	0.18725	0.23275	0.28709	0.1342	0.16138	0.13706	0.18898	0.15611	0.12163	0.04004	0			
13. Fouras	0.0004	0.00162	0.00096	0.00611	0.01656	0.04351	0.02394	0.03502	0.00196	0.00096	0.03104	0.26425	0		
14. Arcachon	0.0032	0.00323	0.00014	0.00443	0.00763	0.03266	0.01404	0.02941	0.00153	0.00658	0.01955	0.22606	0.00145	0	
15. Thau	0.96208	0.95559	0.95617	0.95856	0.94988	0.95236	0.95001	0.94928	0.95614	0.9547	0.9484	0.94167	0.95959	0.95541	0

Fig. 1. Median-joining haplotype network. Each circle represents a haplotype, which frequency is proportional to circle diameter (legend: bottom right). Distances between haplotypes are proportional to the number of mutation events (see text). The Atlantic haplogroup contains one Mediterranean population, represented by haplotype H26 (marked with **).



1
2
3
4
5
6
7 607 Fig. 2. Distribution of haplotype frequencies along the French coast. Abbreviations for site
8 608 names are detailed in Table 1. Haplotype colors correspond to the colors used in Figure 1. The
9
10 609 stations where both *Ocenebra erinacea* and *O. inornatus* were observed
11
12 610 are marked with an asterisk. Map constructed with R package marmap (Pante & Simon-
13
14 611 Bouhet 2013).



Formatted: Font: (Default) Times New Roman