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Phylogeographic study of the dwarf oyster, Ostreola stentina, from Morocco, Portugal and Tunisia: evidence of a geographic disjunction with the closely related taxa, Ostrea aupouria and Ostreola equestris

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Abstract:

Despite the economic importance of oysters due to the high aquaculture production of several species, the current knowledge of oyster phylogeny and systematics is still fragmentary. In Europe, Ostrea edulis, the European flat oyster, and Ostreola stentina, the Provence oyster or dwarf oyster, are both present along the European and African, Atlantic and Mediterranean, coasts. In order to document the relationship not only between O. stentina and O. edulis, but also with the other Ostrea and Ostreola species, we performed a sequence analysis of the 16S mitochondrial fragment (16S rDNA: the large subunit rRNA-coding gene) and the COI fragment (COI: cytochrome oxidase subunit I). Oysters were sampled from populations in Portugal (two populations), Tunisia (two populations) and Morocco (one population), identified as O. stentina on the basis of shell morphological characters. Our data supported a high degree of differentiation between O. stentina and O. edulis and a close relationship between O. stentina and both Ostrea aupouria (from New Zealand) and Ostreola equestris (from Mexico Gulf/Atlantic). The status of this geographic disjunction between these closely related species is discussed. Furthermore, although identified in a separate genus Ostreola by Harry (Veliger 28:121–158, 1985), our molecular data on O. stentina, together with those available for the other two putative congeneric species, O. equestris and Ostreola conchaphila, would favour incorporation of Ostreola in Ostrea. Finally, a PCR-RFLP approach allowed the rapid identification of O. edulis and O. stentina.

Keywords: Phylogeographic study ; Ostrea aupouria ; Ostreola equestris ; Ostreola stentina ; Marocco ; Portugal ; Tunisia

Introduction

Oysters are among the most familiar of all marine invertebrate taxa. However our knowledge of oyster phylogeny and systematics is fragmentary. This is principally due to the plastic growth patterns of these animals, which result in a wide range of overlapping, ecophenotypic variants (Ranson, 1951; Quayle, 1988, Yamaguchi, 1994) that greatly reduce the value of analysis based on shell morphology. Besides that, many intentional or accidental anthropogenic transfers have emphasised this situation. Our current knowledge of oyster phylogeny and systematics is particularly limited for the subfamily *Ostreinae*, encompassing the flat oysters. In spite of the comprehensive reclassification of living oysters made by Harry (1985), numerous cases remained controversial until the use of DNA molecular methods that allowed the independent testing of pre-existing hypotheses. Concerning the *Ostreinae* in general, a major advance was realised by the phylogenetic analysis of Southern hemisphere flat oysters based on 16S sequences by Jozefowicz and O'Foighil (1998). Three ostreinid mitochondrial clades were evident, that were however incongruent with prevailing morphologically-based interpretations of systematic relationships among *Ostreinae*. More recently, O'Foighil and Taylor (2000) showed that the brooding character originated once in the *Ostreinae*, and has been retained in all descendent lineages, providing novel insights into oyster evolution and systematics.

In several cases of misclassification or misidentification of oysters, DNA molecular data, have provided valuable new insights. Hence, different oyster taxa initially described in separate geographical areas have since been grouped as a single species. Recently, Kenchington et al. (2002) suggested that *O. edulis* and *O. angasi* are conspecific. Conversely, DNA tools were used to correct misidentification of species

and to confirm or revise their geographical range. For example, O'Foighil et al. (1999) confirmed the transoceanic range (New Zealand and Chile) of *Ostrea chilensis* using mitochondrial COI sequence data and proposed that dispersal by rafting was the most likely explanation for this distribution. Another example is the occurrence of the European flat oyster, *O. edulis*, in south-western Western Australia, where *O. angasi* was supposed to be the only *Ostreinae* species present (Morton et al., 2003).

All over the world, numerous species (30 to 40 according to the classifications) of oysters of the genus *Ostrea* have been described. Their geographical range is particularly wide in warm and temperate waters of all oceans, with however a predominant tropical distribution (Jaziri, 1990). In Europe, along the Atlantic and Mediterranean coasts, the European flat oyster, *O. edulis*, is the principal flat oyster species and an important economical marine resource. Hence, in 2003 almost 5200 tons were produced in the world, mainly (99%) in Europe (Spain, France, Ireland, Netherlands, United Kingdom), representing about 28 millions euros in value (FAO, 2003). However, another species, *Ostreola stentina* (Payradeau, 1826), also known as the Provence oyster, or dwarf oyster, is also present along the same coasts, and on the Southern Mediterranean coasts and southwestern coast of the Iberian Peninsula, but also along African Atlantic coasts as far as South Africa. Ranson (1967) also indicated its presence along the South coast of Argentina and the Southeastern coasts of Australia, suggesting its ability to grow in waters with very different water temperatures. Several subspecies were also described in Mediterranean Sea as *Ostreola stentina isseli* and *O. stentina pepratxi* by Bucquoy et al. in 1889, or *O. stentina syrica* by Pallary in 1933 but can be considered as synonymous names (Ranson, 1967). Similarly, *O. parenzani*, *O. curvata*, *O. cristata* or *O. plicata*, previously described as different species, can be considered as one, *O. stentina* (Ranson, 1967). Because of the particularly low maximal

shell size of these oysters, between 40 and 50 mm at adult stage, *O. stentina* was not considered as a potential target for aquaculture and studies remain scarce. However, in the context of sympatry of these two species in Spain, *O. stentina* and *O. edulis* larvae were studied in order to determine the temporal occurrence of larvae from both species and, consequently, the settlement period (Pascual, 1972). During a survey of oysters carried out in Portugal in Tejo, Sado, and Mira estuaries, as well as Albufeira lagoon, and Algarve coast, aiming the identification of existing species and their eventual culture, Leal (1984) noted the abundant presence of *O. stentina* in Tejo and Sado estuaries. Then, allozymic markers allowed to clearly distinguish between the two sympatric species present in the Nador Lagoon, in Morocco (Blanc et al., 1986), in the Bay of Cadiz, in Spain, Mira estuary, in Portugal (Amezcua et al., 2001), and Mar Menor Lagoon, in Spain (Gonzalez-Wangüemert et al., 2004). More recently, a molecular analysis based on a centromeric satellite DNA family clearly supported a high degree of differentiation between *O. edulis* and *O. stentina* (Lopez-Flores et al., 2004).

The principal aim of our study was to document the genetic relationship between *O. stentina* and other species of the genera *Ostrea* and *Ostreola*. The second one was to establish a rapid PCR-based method to differentiate easily *O. stentina* and *O. edulis*. Therefore, we performed sequence analysis of the 16S mitochondrial fragment (16SrDNA: the large subunit rRNA-coding gene) and the COI fragment (COI: Cytochrome Oxidase subunit I) on flat oysters individuals sampled in Portugal, Tunisia and Morocco, identified as *O. stentina*, on the basis of morphological characters. We compared the sequences obtained with the other *Ostrea* and *Ostreola* sequences

published or submitted in databanks and then more especially with *O. edulis* in order to establish the differentiating PCR-RFLP method between the two species.

Material and methods

Biological Samples

A total of 214 dwarf oysters were sampled in 2003 from five locations, two in Tunisia (Gulf of Gabès), two in Portugal (Sado and Mira estuaries), and one in Morocco (Dakhla Bay) (Figure 1). In Tunisia, two sampling were performed, one in front of the Kneiss Islands (34° 25’ N, 10° 10’ E) abbreviated KN and one in Ghannouche (34° 01’ N, 9° 53’ E) abbreviated GH, with respectively 74 and 8 dwarf oysters. In Tunisia, the oysters were found aggregated and low-lying in the mudbank. In Portugal, 13 oysters were collected in Sado estuary (SA: 38° 25’ N, 8° 39’ W) and 101 oysters in Mira estuary (MI: 37° 42’ N, 8° 44’ W). In Morocco, 18 oysters were collected in the Dakhla Bay (MO: 23° 42’ N, 15° 57’ O).

The morphological identification was performed on the basis of Harry’s criteria (1985) : (1) presence of chomata, (2) adductor muscle scar discoloured, (3) small size (less than 40 mm), (4) height greater than width, (5) no lamellae. Furthermore, and in parallel of the DNA sampling for molecular analyses, a biometric analysis (shell length) was performed on 1399 oysters sampled every 15 days during 9 months in Tunisia, allowing to sample the different classes of size. The same kind of measurement was performed on the 114 Portuguese oysters sampled for the molecular analyses.

Furthermore, one sample of *Ostrea conchaphila* originating from Washington State, USA, was added to our COI sequencing analysis.

142 Amplification and sequencing

143 DNA extraction of ethanol-preserved gill fragments was performed by a
144 phenol/chloroform method, as described by Moore (1993). We amplified the 16S
145 mitochondrial fragment with primers described by Banks et al. (1993), according to the
146 protocol detailed in Boudry et al. (1998). A partial COI fragment was also amplified
147 using the primers and conditions detailed in Folmer et al. (1994). The PCR products
148 were purified with a High Pure PCR Product Purification Kit (Boehringer-Mannheim,
149 Germany). The sequencing reaction, consisting of a first step of denaturation (2 min,
150 92°C) and 30 cycles (30 s, 95°C, denaturating; 30 s, 50°C, annealing; 1 min, 72°C,
151 elongation), was performed with the Sequitherm EXCEL™ II DNA sequencing kit-LC
152 (Epicentre Technologies). The fragments were separated on a Li-Cor® 4200 automated
153 DNA sequencer. All the novel sequences were submitted to the Genebank nucleotide
154 sequence database.

155

156 DNA sequence analysis

157 The 16S sequences obtained, together with some sequences already obtained by
158 Jozefowicz and O'Foighil (1998) for *Ostrea edulis*, *Ostreola conchaphila*, *Ostrea*
159 *puelchana*, *Ostrea denselamellosa*, *Ostrea chilensis*, *Ostrea aupouria*, *Ostrea angasi*,
160 *Ostrea algoensis*, *Ostreola equestris* (corresponding to *Teseyostrea weberi* in their
161 paper) - whose accession numbers are respectively AF052068, AF052071, AF052073,
162 AF052067, AF052065, AF052064, AF052063, AF052062, AF052074 - were aligned
163 with CLUSTALW (Thompson et al. 1994). The same procedure was applied for COI
164 sequences obtained, together with some sequences already obtained by Giribet and
165 Wheeler (2002) for *Ostrea edulis*, O'Foighil et al. (1999) for *Ostrea chilensis*, *Ostrea*
166 *aupouria*, *Ostrea angasi*, and Kirkendale et al. (2004) for *Ostreola equestris* - whose

accession numbers are respectively AF120651, AF112289, AF112288, AF112287, AY376607. Pairwise sequence divergences between species were estimated with the DNADIST program in PHYLIP (Felsenstein, 1989, http://evolution.genetics.washington.edu/phylip/felsenstein.html) according to Kimura's two-parameter model (Kimura, 1980). Phylogenetic analyses were conducted using the program NEIGHBOR in PHYLIP. Bootstrap analysis with 100 replicates was performed with the SEQBOOT and CONSENSE programs in PHYLIP.

PCR-RFLP

According to the alignment of the *O. stentina* and *O. edulis* 16S sequences, two polymorphic sites were identified as candidate for restriction analysis as commercial digestion enzymes, *Rsa*I and *Tru9*I, are available at these positions. Among them, for reading convenience, the restriction with *Rsa*I was preferentially chosen as only none (*O. stentina*) or one (*O. edulis*) site was present in our sequences, instead of respectively 6 and 9 for *Tru9*I. The digestion reaction was performed at 37°C during 90 min with 10 µl of PCR product and 10 µl of a mix encompassing 10X buffer and 3 units *Rsa*I enzyme. The resulting fragments were separated on 1% agarose gels and stained with ethidium bromide.

Results

Species identification

The biometric analysis on the Tunisian samples showed that the mean length of these oysters was 26 mm but never more than 47 mm. In Portugal, the oysters had a mean length of 29 mm (range of 21-38 mm) in Sado estuary and of 37 mm (range of 23-52 mm) in Mira estuary. In Morocco, the oysters were not measured when sampled but

described as “small”. According to the presence of the 5 criteria described by Harry (1985) and the biometric analyses, all these oysters were identified as *O. stentina*.

Sequence analysis

Three new different 16S sequences were detected encompassing respectively and without any exception samples from Tunisia, Portugal, and Morocco. These sequences were respectively registered as Accessions DQ13178 for GH - KN, DQ13179 for MI – SA, and DQ13180 for MO. The alignment of these new sequences with the other *Ostrea* species sequences allowed the reconstruction of a neighbor-joining tree presented in Figure 2. The genetic distances (Table 1) confirmed that all the *O. stentina* samples we studied are genetically very close, but surprisingly also with the *O. aupouria* and *O. equestris* samples sequenced by Jozefowicz and O’Foighil in 1998. Hence the divergence between the three *O. stentina* sequences is always below 2% with 1.8 % between GH – NK and both MI – SA and MO. The Tunisian sequence is more differentiated from the Portuguese and Moroccan sequences than these two later are with an under estimation of 0% divergence between them (as the estimator does not take into account the single indel difference). The divergence is also very low between the Tunisian, Portuguese or Moroccan *O. stentina* sequences and *O. aupouria*, ranging from 1.3 to 1.6% according to the population of *O. stentina* considered. The same low divergence is observed between the *O. stentina* sequences and *O. equestris*, between 1.3 and 1.5%. Besides, the divergences between these *O. stentina* samples and all the other *Ostrea* species sequences are raising between 3 and 9% with a clear differentiation with *O. edulis* (between 7 and 8.2% divergence).

This close relationship between our 5 populations of *O. stentina* and *O. aupouria* and *O. equestris* is confirmed by our analysis of the three *O. stentina* COI sequences (Accessions DQ13181Q for GH - NK, DQ13182 for MI – SA, and DQ13183 for MO; Table 2, Figure 3). The COI divergence is also very low between *O. aupouria* and the Tunisian, Portuguese or Moroccan *O. stentina* sequences, ranging from 4.0 to 4.7%, but also with *O. equestris*, ranging from 3.3 to 4.2%. The divergences between these *O. stentina* samples and all the other *Ostrea* species sequences range from 14.9 to 24.1% with a clear differentiation with *O. edulis* (between 21.7 and 22.5% divergence). The new COI sequence for *O. conchaphila* was registered as Accession DQ464125.

Rapid detection of *O. stentina* specimens

Thanks to the PCR-RFLP analysis performed with the 16S-R*saI* fragment-enzyme couple, *O. stentina* samples can be easily identified on agarose gels as they present one band (no restriction site), conversely to *O. edulis* samples that present two bands (one restriction site) (Figure 4). All the 214 samples proved to be *O. stentina*.

Discussion

Ostrea edulis and *Ostreola stentina* both inhabit Southwestern coast of the Iberian Peninsula, Mediterranean Sea and African Atlantic coasts and they are sympatric in some areas (Leal, 1984)). In Nador Lagoon (Morocco), a cohort of 98 oysters supposed to be *O. edulis*, with 49 individuals characterised as fast-growing and 49 as slow-growing animals, were studied with allozymic markers (Blanc et al., 1986). The authors concluded that the fast-growing sample was *O. edulis*. Of the slow-growing oysters, only 19% were considered to be *O. edulis*, while 81% belonged to another species. Hence, the external morphology of *O. edulis* and *O. stentina* is very similar and this

241 prevents their differentiation at the morphological level, especially when the oysters are
242 small. *O. edulis* can reach 94 g and 95 mm in weight, and size respectively after thirteen
243 months in culture, whereas *O. stentina* does not exceed 20g in weight and 45 mm in size
244 (Rosique et al., 1995). This is in agreement with our biometric analysis performed on
245 the Tunisian flat oysters, that showed a mean length of 26 mm but never more than 47
246 mm, and on the Portuguese flat oysters, that showed a mean length of 29 mm in Sado
247 estuary and of 37 mm in Mira estuary. This is also in agreement with recent hatchery
248 experiments (F. Batista, perso. com.) that showed that *O. stentina* samples can reach at
249 best 20 mm size in approximately 3 months. However, the potential range of
250 overlapping between both species until the 40-50 mm would make another criteria
251 useful for their distinction.
252 Allozymic markers were the first to clearly distinguish between the two sympatric
253 species present in the Nador Lagoon, in Morocco (Blanc et al., 1986), in the Bay of
254 Cadiz in Spain and in Mira estuary in Portugal (Amezcua et al., 2001), and in the Mar
255 Menor Lagoon, in Spain (Gonzalez-Wangüemert et al., 2004). Our molecular DNA
256 analysis confirm this statement as the individuals of the five populations of small flat
257 oysters identified as *O. stentina* in Morocco, Portugal and Tunisia, showed a very
258 different sequence from *O. edulis* samples for both 16S (between 7 and 8.2%
259 divergence) and COI (between 21.7 and 22.5% divergence) fragments, this latter being
260 a faster-evolving mt gene fragment than the former. As *O. edulis* may still represent a
261 high commercial value in Europe (the value of farmed *O. edulis* production in 2002 was
262 US$ 24.3 million), its easy distinction with other sympatric species in Europe is of
263 particular interest. Therefore, our easy PCR-RFLP technique allows a simple distinction
264 between *O. edulis* and *O. stentina* (Figure 4).
265

More surprising may be the very close relationship between *O. stentina* and both *O. aupouria* and *O. equestris*: 1.3 to 1.5% divergence between *O. stentina* and each of *O. aupouria* and *O. equestris* for 16S, and 4 to 4.7% and 3.4 to 4.5% divergence between *O. stentina* and *O. aupouria*, and *O. equestris* respectively for COI. This is particularly true between *Ostreola stentina* and *Ostrea aupouria* as they don't belong to the same genus, although *Ostreola stentina* and *Ostreola equestris* do. Furthermore, *O. aupouria* and *O. equestris* can be found respectively in New Zealand and on the coasts of Florida (United-States), both on the Atlantic side and Mexico gulf side as well as the Florida Keys. *O. aupouria* can be distinguished from the co-occurring and predominant *O. chilensis* by the anal appendage (Dinamini and Beu, 1981). *O. equestris*, commonly known as the "crested" oyster, is described as having a shell with raised crenulated margins (Abbott, 1974). Kirkendale et al. (2004) found a very close relationship between these two species with COI sequences analysis. Our study introduces a third species, *Ostreola stentina*, in this geographic disjunction of three closely related species. This observation is but one of three such cases involving taxa in the brooding oyster 16S gene tree with *Ostrea edulis*/*Ostrea angasi* and *Cryptostrea permollis*/*Ostrea puelchana* first discussed in Jozefowicz and O'Foighil (1998). Furthermore, on the basis of a repeat region in ITS-1, Kenchington *et al*. (2002) suggested that *Ostrea edulis* and *O. angasi* are conspecific. In the case of *Ostreola equestris*/*Ostrea aupouria* geographic disjunction, Kirkendale et al. (2004) ruled out historic transfers that have occurred or have been suspected on numerous occasions (Dinamini, 1971; Edwards, 1976; Buroker et al., 1979; Chew, 1990; Carlton and Mann, 1996; Boudry et al., 1998; O'Foighil et al., 1998; Lapègue et al., 2002), as possible origin of this disjunction observation. Indeed, their study of several populations in both species allowed them to conclude that New Zealand *Ostrea aupouria* and Gulf/Atlantic

Ostreola equestris are reciprocally monophyletic. This, together with their lack of shared COI haplotypes, is characteristic of populations that have not experienced recent gene flow (Avise, 2000) and so no anthropogenic transoceanic introductions. In our case, a deeper comparison with the COI marker or even more polymorphic regions, between our *Ostreola stentina* populations and respectively *Ostreola equestris* and *Ostrea aupouria* ones is needed to allow the inference of the origin of such a geographic disjunction between our Mediterranean/African-Atlantic species and the two others. However, a recent palaeontological study gives evidence for an old presence of *Ostreola stentina* in Southwestern coast of the Iberian Peninsula (T. Drago, perso. com.), as the author identified, according to Harry's (1985) criteria, *O. stentina*-like shells in Algarve (South Portugal) aged of more than 6000 years.

As observed by Kirkendale et al. (2004), *Ostreola equestris*, *Ostreola conchaphila,* and now *Ostreola stentina*, the three constituent species of *Ostreola* genus, could not be considered as sister taxa in our 16S or COI trees. Hence, although *Ostreola conchaphila* is closer to the *Ostreola stentina*, *Ostreola equestris*, and *Ostrea aupouria* group (3 to 4.2%) than to the other species (5.5 to 8%) for the 16S fragment, it is not as close as the *Ostreola stentina*, *Ostreola equestris*, *Ostrea aupouria* are among each other (0.8 to 1.6%). Again, although *Ostreola conchaphila* is closer to the *Ostreola stentina*, *Ostreola equestris*, and *Ostrea aupouria* group (14.9 to 16%) than to the other species (19.2 to 22.3%) for the COI fragment, it is not as close as the *Ostreola stentina*, *Ostreola equestris*, *Ostrea aupouria* are among each other (1.3 to 5.3%). Therefore, although identified in a separate genus *Ostreola* by Harry (1985), our molecular data agree with those of Kirkendale et al. (2004) and would favour incorporation *Ostreola* in *Ostrea* as proposed by Coan et al. (2000). However, without considering the taxonomic

316 debate, it clearly appears that *O. stentina*, *O. aupouria* and *O. equestris* have to be
317 considered as a particular group in the Ostreinae, and deserve particular attention, as it
318 could, with its particular geographic disjunction in New Zealand, Mexico Gulf/Atlantic
319 and Mediterranean/African-Atlantic. This could help understanding a counter-subject of
320 the phylogeny of oysters and more specially the Ostreinae one's.
321

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LEGENDS OF THE FIGURES

Figure 1. Location of the samples : Gannouche (GH), Kneiss (KN) in Tunisia, Mira (MI) and Sado (SA) for Portugal, (MO) for Morocco.

Figure 2. Phylogenetic tree obtained from sequence divergence of a 489 bases nucleotide mitochondrial 16S DNA fragment according to Kimura's model (Kimura 1980) for 3 sequences of *O. stentina*, 9 sequences by Jozefowicz and O'Foighil (1998) for *O. edulis*, *O. conchaphila*, *O. puelchana*, *O. densalamellosa*, *O. chilensis*, *O. aupouria, O. angasi*, *O. alogensis* , and *O. equestris*. Numbers on the branches indicate bootstrap values superior to 50%.

Figure 3. Phylogenetic tree obtained from sequence divergence of a 660 bases nucleotide mitochondrial COI fragment according to Kimura's model (Kimura 1980) for 3 sequences of *O. stentina*, 1 sequence of *O. conchaphila*, obtained in this study, for 1 sequence by Giribet and Wheeler (2002) for *Ostrea edulis*, for 3 sequences by O'Foighil et al. (1999) for *Ostrea chilensis*, *Ostrea aupouria*, *Ostrea angasi*, and 1 sequence by Kirkendale et al. (2004) for *O. equestris*. Numbers on the branches indicate bootstrap values superior to 50%.

Figure 4. Example of a rapid PCR-RFLP identification on agarose gel of *Ostreola stentina* samples (2 bands in columns 6, 7, and 8) and *Ostrea edulis* samples (1 band in columns 2, 3, 4, 10, 11, 12) with the restriction by *Rsa*I of 16S fragments. Columns 1, 5, 9, and 13 correspond to the 1kB ladder.

461 Table 1. Pairwise sequence divergences, for the mt 16SrDNA fragment.

462

	Ostrea puelchana	*Ostrea chilensis*	*Ostreola conchaphila*	*Ostrea denselamellosa*	*Ostrea edulis*	*Ostrea angasi*	*Ostrea algoensis*	**GH – KN**	**MI - SA**	**MO**	***Ostreola equestris***
Ostrea chilensis	0.0438										
Ostreola conchaphila	0.0273	0.0579									
Ostrea denselamellosa	0.0438	0.0676	0.0554								
Ostrea edulis	0.0683	0.0486	0.0754	0.0804							
Ostrea angasi	0.0732	0.0533	0.0803	0.0853	0.0045						
Ostrea algoensis	0.0663	0.0685	0.0710	0.0637	0.0684	0.0733					
GH – KN	0.0400	0.0619	0.0424	0.0676	0.0823	0.0881	0.0799				
MI - SA	0.0317	0.0562	0.0396	0.0642	0.0822	0.0880	0.0827	**0.0185**			
MO	0.0303	0.0520	0.0350	0.0615	0.0697	0.0747	0.0727	**0.0185**	**0.000**		
Ostreola equestris	0.0344	0.0603	0.0296	0.0602	0.0729	0.779	0.0659	**0.0129**	**0.0156**	**0.0138**	
Ostrea aupouria	0.0371	0.0616	0.0363	0.0586	0.0819	0.0877	0.0796	**0.0156**	**0.0129**	**0.0131**	**0.0077**

463

464 Table 2. Pairwise sequence divergences, for the mt COI DNA fragments.

465

	Ostrea chilensis	*Ostrea edulis*	*Ostrea angasi*	*Ostreola conchapila*	**GH – KN**	**MI - SA**	**MO**	***Ostreola equestris***
Ostrea edulis	0.1331							
Ostrea angasi	0.1370	0.0182						
Ostreola conhaphila	0.2229	0.1941	0.1916					
GH – KN	0.2300	0.2249	0.2404	0.1488				
MI - SA	0.2324	0.2233	0.2408	0.1599	**0.0536**			
MO	0.2150	0.2176	0.2330	0.1514	**0.0439**	**0.0087**		
Ostreola equestris	0.2112	0.2131	0.2182	0.1489	**0.0337**	**0.0452**	**0.0419**	
Ostrea aupouria	0.2137	0.2099	0.2185	0.1537	**0.0439**	**0.0474**	**0.0400**	**0.0132**

Figure 1

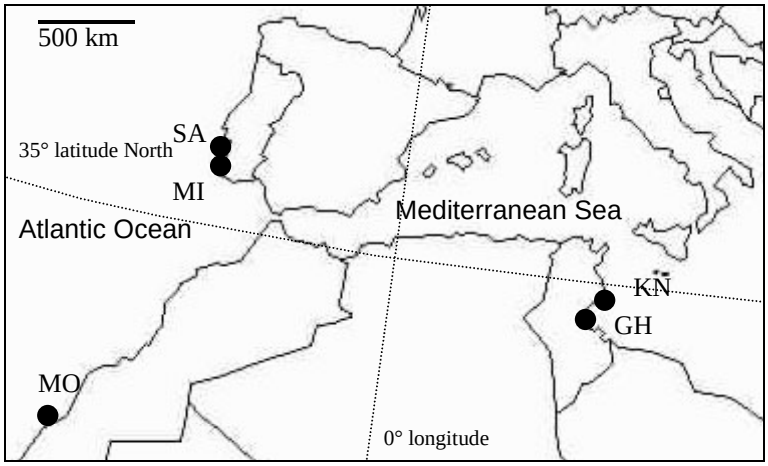
500 km
SA
35° latitude North
MI
Atlantic Ocean
Mediterranean Sea
KN
GH
MO
0° longitude

Figure 2

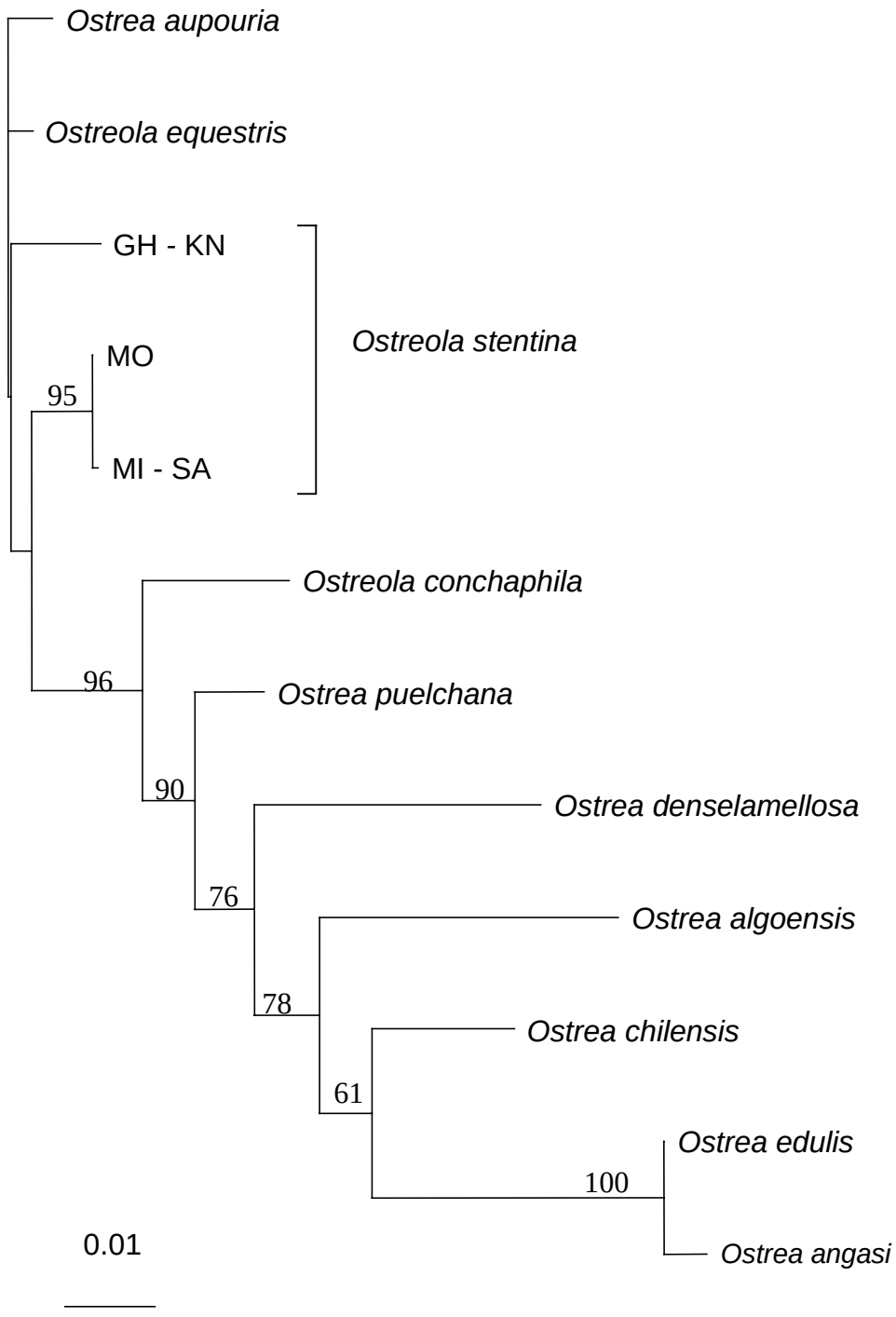
Ostrea aupouria
Ostreola equestris
GH - KN
MO
95
MI - SA
Ostreola stentina
Ostreola conchaphila
96
Ostrea puelchana
90
Ostrea denselamellosa
76
Ostrea algoensis
78
Ostrea chilensis
61
Ostrea edulis
100
Ostrea angasi
0.01

Figure 3

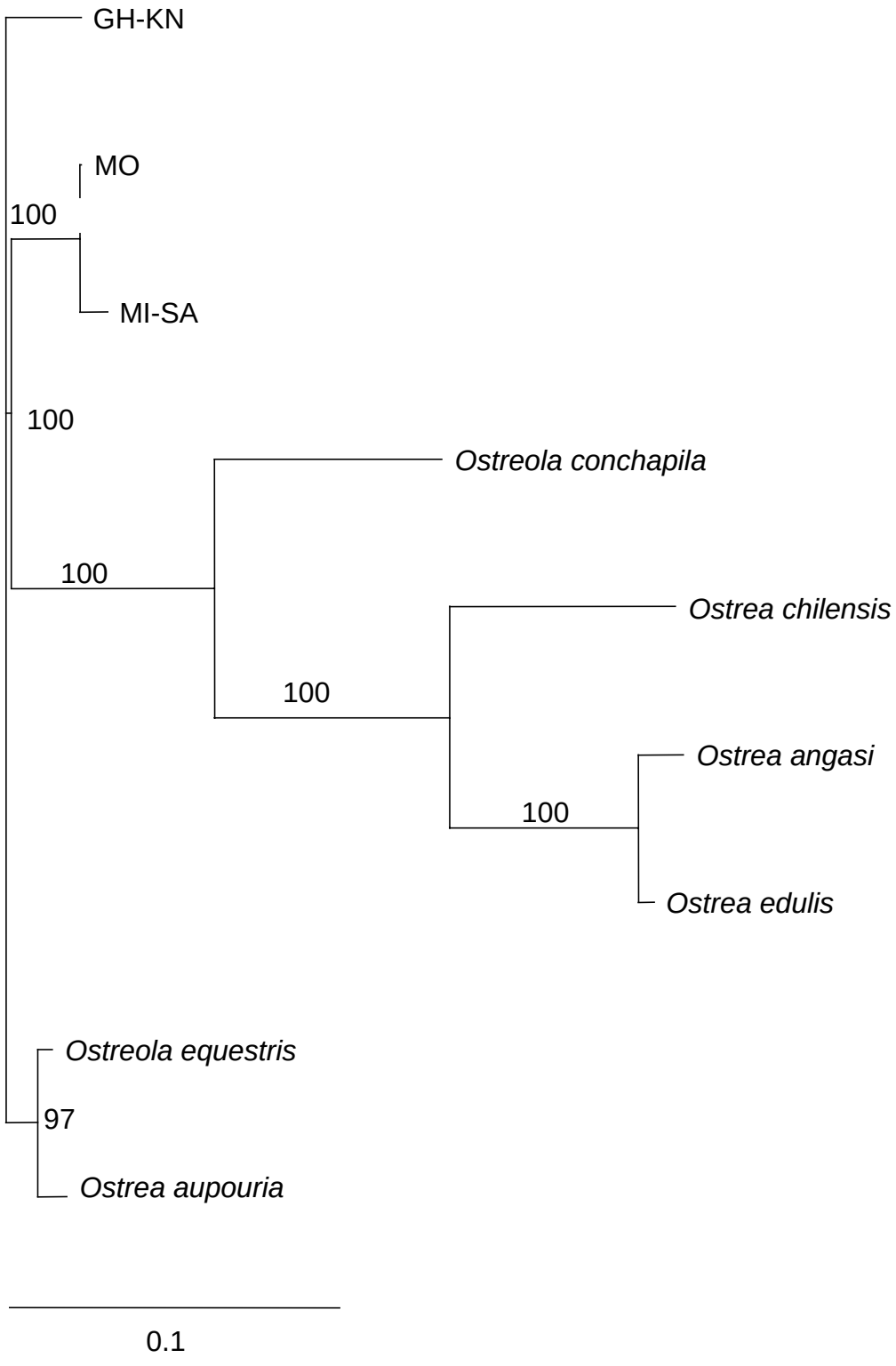
GH-KN
MO
100
MI-SA
100
Ostreola conchapila
100
Ostrea chilensis
100
Ostrea angasi
100
Ostrea edulis
Ostreola equestris
97
Ostrea aupouria
0.1

Figure 4