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# Trans-Atlantic Distribution of a Mangrove Oyster Species Revealed by 16S mtDNA and Karyological Analyses

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**Abstract.** Three species of mangrove oysters, *Crassostrea rhizophorae, C. brasiliana,* and *C. gasar,* have been described along the Atlantic shores of South America and Africa. Because the distribution of these molluscs is of great biological and commercial interest, their taxonomy and distribution deserve further clarification. Therefore, 15 populations were sampled from both continents. Their 16S mitochondrial polymorphism was studied by sequencing and PCR-RFLP analysis. Two haplotypes were identified. Haplotype a was the only one observed in Africa, but it was also observed in South America together with haplotype b. Because *C. gasar* is the only mangrove oyster identified on the west coast of Africa, haplotype a was attributed to this species, which has thus been shown to occur in South America. Haplotype b is attributed to *C. rhizophorae*. The karyotypes of specimens of *C. gasar,* from Africa and from South America, were very similar, and both species were observed at the same location in Brazil. The occurrence of *C. gasar* in South America adds a third species—in addition to *C. rhizophorae* and *C. brasiliana*—to the list of species present along these coasts. The predominant surface circulation patterns in this part of the Atlantic Ocean favor the hypothesis that *C. gasar* was transported from Africa to America. Finally, a phylogenetic tree built with seven 16S sequences from *Crassostrea* and *Saccostrea* species showed that *C. gasar* is intermediate between the American *Crassostrea* species (*C. virginica* and *C. rhizophorae*) and the Asian species (*C. gigas* and *C. ariakensis*).

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## Introduction

Mangrove ecosystems are widely distributed; they cover 100,000–200,000 km$^2$ of the world's tropical estuarine zones where sea and rivers mix (Blasco *et al.,* 1998). The mangrove trees characterize these ecosystems and constitute a natural habitat for mangrove oysters; the aerial prop roots of the trees provide the oyster larvae with a convenient place to settle in the intertidal zone. Because mangrove oysters live naturally on mangrove roots, which are called rhizophores, the latter term was used in the taxonomic name of a South American mangrove oyster, *Crassostrea rhizophorae* (Guilding, 1828). In fact, numerous species of mangrove oysters have been described, all in the genus *Crassostrea*; but the taxonomic identification is difficult and uncertain, so their geographical ranges are also often poorly known.

These problems and uncertainties are well illustrated by the mangrove oysters of South America and Africa. The taxonomic status of the oysters growing along the Atlantic coast of South America has been widely investigated morphologically, ecologically, physiologically, and genetically. Some authors have regarded the subtidal rocky shore form of *C. rhizophorae* (Guilding, 1828) as distinct, mainly because of its large size (*e.g.,* Nascimento, 1991), and have applied to it the binomen *C. brasiliana* (Lamarck, 1819).

But because size is considered unreliable as a taxonomic character, *C. brasiliana* was held by Rios (1994) to be synonymous with the generally smaller *C. rhizophorae*. However, large differences in growth rates and larval morphology have been described between *C. rhizophorae* and *C. brasiliana* populations, suggesting that they may indeed be distinct biological species (Absher, 1989). Moreover, their geographic range appears to be different: *C. brasiliana* occurs on the Caribbean coast of South America, whereas *C. rhizophorae* is more common and is found from Florida to Brazil (Littlewood, 1991). Finally, the occurrence of two distinct species along the South American coasts was clearly demonstrated recently by an allozyme study (Ignacio *et al.,* 2000). *C. rhizophorae* is now extensively cultivated throughout the various countries of the Caribbean Sea, as well as in the West Indies, and is considered to be a commercially important species (Arakawa, 1990). Moreover, *C. rhizophorae* is also being produced in New Guinea (FAO, 1999).

Oysters from the coasts of Africa have been less extensively studied than those from South America (Marozova *et al.,* 1991). Although *C. cucculatta* (Born) is the only species described from the eastern coast of Africa (and Madagascar), two species names are used for oysters growing along the western coast: *C. gasar* (Adanson) and *C. tulipa* (Lamarck). *C. gasar* has been reported in Mauritania (Gowthorpe, 1993), Senegal and Gambia (Diop, 1993), Ivory Coast (Egnankou, 1993), Nigeria (Isebor and Et Awosika, 1993), and Cameroon (Zogning, 1993). Two different names were given in Togo: "*Gryphea*" or *C. gasar* (Akpagana, 1993); and in Congo: *Gryphea gasar* (*Crassostrea tulipa,* Lamarck) (Makaya, 1993). The *C. tulipa* species name is also mentioned in Liberia (Yoo and Ryu, 1984) and in Sierra Leone (Kamara, 1982). As *C. tulipa* is now considered a synonym of *C. gasar* (Marozova *et al.,* 1991), we will use this name for samples collected from the south Atlantic African coast. *C. gasar* is a commercially important bivalve in Africa (Nicklès, 1950)—for example, in Nigeria (Ajana, 1979) and Senegal (Cormier-Salem, 1987)—and its potential for more intensive aquacultural production has been studied (Cormier-Salem, 1987; Marozova *et al.,* 1991).

A typical feature of oysters from the genus *Crassostrea* is the extreme variability of the shell (Galtsoff, 1964). Moreover, this variability also extends to the soft tissues (Lawrence, 1995). Therefore, oysters are often difficult to differentiate on the basis of their morphology. Consequently, other methods, such as karyological and molecular analyses, must be applied to distinguish the different mangrove oyster species. A study of seven species of cupped oyster showed that the karyotype of *C. gasar* is clearly isolated from two other groups, one composed of *C. gigas, C. angulata,* and *C. sikamea,* and the other of *C. virginica, C. ariakensis,* and *Saccostrea commercialis* (Leitão *et al.,* 1999). The karyotype of *C. rhizophorae* has also been previously reported in specimens from Mexico (Rodriguez-Romero *et al.,* 1979; Ladron de Guevara *et al.,* 1996) and from Venezuela (Marquez, 1992), and it appears to be different from those of the species described by Leitão *et al.* (1999).

Molecular methods can usefully complement morphological and karyological studies in determining the status of oyster taxa. For example, such methods have already been used to infer the phylogenetic relationships among species of cupped oysters (Littlewood, 1994), to discriminate between closely related Asian *Crassostrea* species (Banks *et al.,* 1993; Hedgecock *et al.,* 1999), to better understand the close relationship between *C. gigas* and *C. angulata* (Boudry *et al.,* 1998; O'Foighil *et al.,* 1998), and to distinguish among sympatric species of the rock oyster *Saccostrea* in Thailand (Day *et al.,* 2000). However, little molecular taxonomy has been done on mangrove oysters: a few genetic studies (allozyme data) have been carried out on *C. rhizophorae* (Hedgecock and Okazaki, 1984; Ignacio *et al.,* 2000), but nothing has been published previously about *C. gasar*.

In this study, the methods of molecular biology and karyology were used to ascertain the taxonomic status of the mangrove species present along the shores of the south Atlantic, and to determine the phylogenetic position of the African species in the *Crassostrea* clade. To these ends, we studied African mangrove oyster samples, described as *C. gasar* or *C. rhizophorae* (W.B. Dambo, Rivers State University of Science and Technology, Nigeria, pers. comm.), and American mangrove oysters, presumed to be *C. rhizophorae* or *C. brasiliana*. In particular, we analyzed the 16S mitochondrial fragment that had already been studied in other species of the genus *Crassostrea* by O'Foighil *et al.* (1995), and also in *Saccostrea* (K.K.Y. Lam and B. Morton, Swire Institute of Marine Science, The University of Hong Kong, China, unpubl. data) and *Ostrea* (Jozefowicz and O'Foighil, 1998). With these data, we could analyze the genetic relationship between *C. gasar* and the other species. We also examined the karyotype of the presumed *C. rhizophorae* samples from French Guiana and compared them with the karyotype of *C. gasar* from Senegal.

## Materials and Methods

### *Sampling*

Ethanol-fixed samples or live mangrove oysters were obtained from wild populations of south Atlantic coasts (see Fig. 1 for locations). Putative *C. rhizophorae* were collected along the Atlantic coast of South America: from Martinique in 1997 (MAS), French Guiana in 1997 (SIN), and Brazil. From this last location, two samplings were made, the first in 1997 (PAR1) on two islands (Ilha Rosa and Ilha das Gambas) inside Paranagua Bay, and the second sampling in 1998 at the harbor of Guaraqueçaba on the border of the

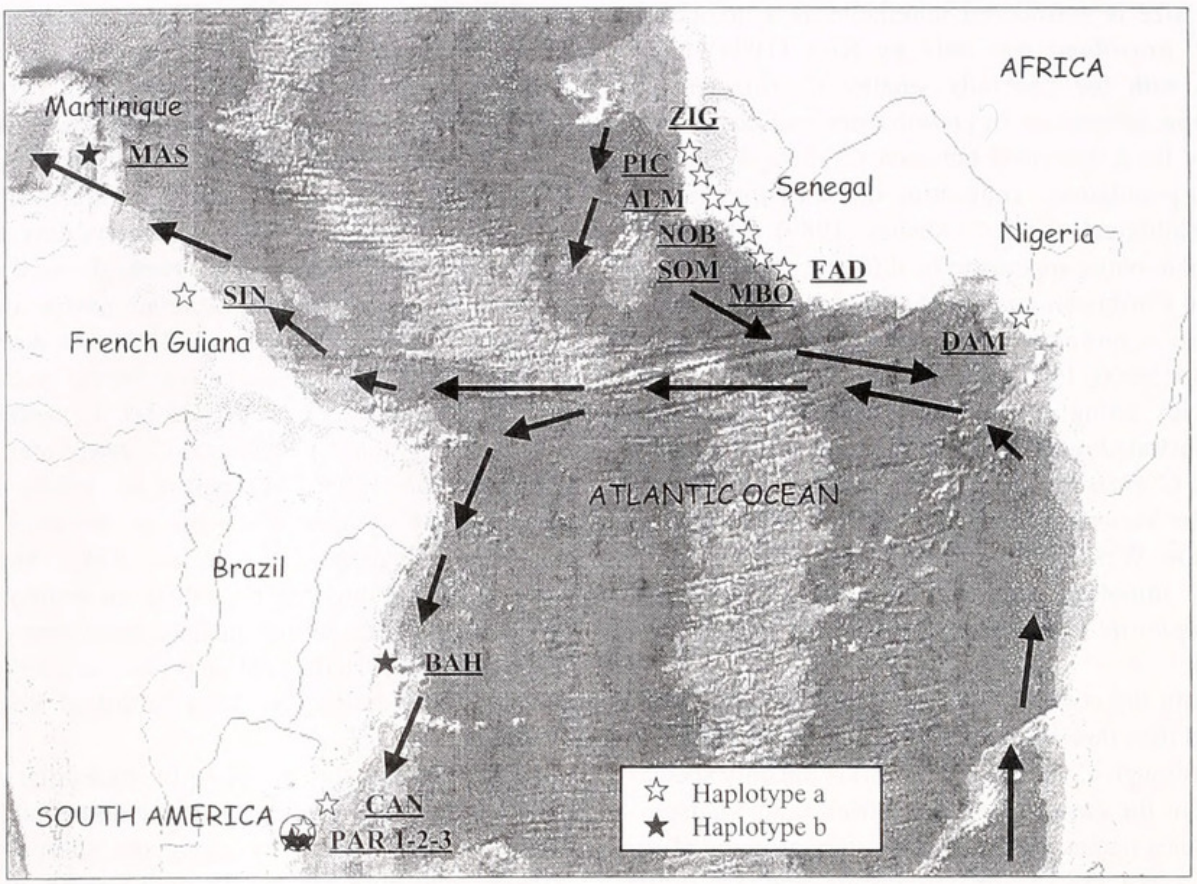


**Figure 1.** Population collection sites and their taxonomic status based on 16S gene analysis. The arrows indicate the predominant surface circulation patterns in this part of the Atlantic Ocean. See legend of Table 1 for details on the three PAR samples.

same bay. In this second sample, two groups (PAR2 and PAR3) were selected on the basis of their size: PAR2 specimens described as "fast growers," and PAR3 specimens described as "slow growers." Two other samples from Brazil were collected in 1999: in the Cananéia Bay (CAN), and near Salvador do Bahia (BAH). Putative *C. gasar* samples were provided in 1999 from locations along the Senegalese coasts (ZIG, NOB, PIC, ALM, SOM, MBO, FAD), and specimens were taken from the Niger estuary (DAM), described as *C. rhizophorae* in Nigeria in 2000. Generally, the samples were collected on either mangrove roots or rocks; but in the Paraguana Bay, they were all sampled on rocks, and in French Guiana on mangrove roots. Table 1 summarizes the characteristics of these samples. Two animals from each of the populations SIN and NOB were chosen for karyological analysis, as they were initially thought to represent *C. rhizophorae* and *C. gasar* respectively.

*Mitochondrial DNA analysis*

DNA extraction of gill fragments was performed either by a Chelex-based method, as described in Estoup *et al.* (1996), or by a phenol/chloroform method, as described by Moore (1993). We amplified the 16S mitochondrial fragment (16SrDNA: the large subunit rRNA-coding gene) with primers described by Banks *et al.* (1993), according to the protocol detailed in Boudry *et al.* (1998).

A first set of samples (two individuals from each of nine populations, as indicated in Table 1) was studied by sequencing the mitochondrial 16S fragment. The PCR products were then purified with a high pure PCR product purification kit (Boehringer-Mannheim, Germany), and manually sequenced with an oligonucleotide tailing kit (Boehringer-Mannheim, Germany) and γ33P radiolabeled deoxynucleotide triphosphate (dNTP). The sequencing reaction, consisting of 35 cycles (30 s, 95°C, denaturating; 30 s, 55°C, annealing; 1 min, 72°C, elongation), was performed according to the manufacturer's instructions.

The samples sequences—together with some sequences already obtained for *C. virginica, C. gigas, C. ariakensis* (O' Foighil *et al.*, 1995), *S. commercialis* and *S. mordax* (K.K.Y. Lam and B. Morton, Swire Institute of Marine Science, The University of Hong Kong, China, unpubl. data, accession numbers AF353099 AF353100), and *O. edulis* (Jozefowicz and O'Foighil, 1998)—were aligned with the software CLUSTALW (Thompson *et al.*, 1994). Parsimony analysis was implemented with PHYLIP (Felsenstein, 1989) using the program DNAPARS. Boot-

**Table 1**

*Characteristics of the populations of* Crassostrea *sampled*

| Population name | Putative species | Location | Number of individuals | DNA sequence | PCR-RFLP haplotype |
|---|---|---|---|---|---|
| ZIG | *C. gasar* | Zinguichor, Senegal | 2 | A | a |
| NOB | *C. gasar* | Kafountine, Senegal | 2 | A | a |
| PIC | *C. gasar* | Senegal | 2 | A | a |
| ALM | *C. gasar* | Almadies, Senegal | 2 | A | a |
| SOM | *C. gasar* | Somone, Senegal | 12 | | a |
| MBO | *C. gasar* | M'Bour, Senegal | 6 | | a |
| FAD | *C. gasar* | Joal, Senegal | 6 | | a |
| DAM | *C. rhizophorae* | Niger estuary, Nigeria | 12 | | a |
| SIN | *C. rhizophorae* | Mont Sinery, French Guiana | 2 | A | a |
| MAS | *C. rhizophorae* | Martinique | 2 | B | b |
| PAR1* | *C. rhizophorae* | Paranagua Bay, Brazil | 2 | A | a |
| PAR2* | *C. rhizophorae* | Paranagua Bay, Brazil | 2 | B | b |
| PAR3* | *C. rhizophorae* | Paranagua Bay, Brazil | 2 | B | b |
| BAH | *C. rhizophorae* | Salvador do Bahia, Brazil | 6 | | b |
| CAN | *C. rhizophorae* or *C. brasiliana* | Cananéia | 6 | | a |

* The three samples PAR were collected in the same area, the Paranagua Bay in Brazil. However, PAR1 was collected in 1997 on two islands inside the bay, and PAR2 and PAR3 in 1998 in a harbor on the border on the bay. Furthermore, the latest two samples differ in size. See Figure 1 for the location of the samples.

strap analysis with 100 replicates was performed by the SEQBOOT and CONSENSE programs from the PHYLIP package. Pairwise sequence divergences between species were estimated by the DNADIST program from PHYLIP according to Kimura's two-parameter model (Kimura, 1980).

With a second set of samples, the same mitochondrial fragment was studied by PCR-RFLP using the appropriate *Taq*I restriction enzyme at 65°C; the particular enzyme selected was determined by the sequence information we obtained. Restriction reactions were performed in a 20-$\mu$l volume composed of PCR product, 1× reaction buffer, and 2–5 units of endonuclease for 2 h. The digested results were resolved after electrophoresis on 1% agarose gels in 1× TBE (Tris–boric acid–EDTA), and stained with ethidium bromide.

*Karyological analysis*

Chromosome preparations were carried out according to Leitão *et al.* (1999). After acclimation in the hatchery of La Tremblade, France, oysters were incubated in a solution of colchicine (0.005%) in seawater. Dissected gills were treated in sodium citrate (0.9%), and then fixed in four successive baths of a freshly prepared mixture of absolute ethanol and acetic acid (3:1). Slides were prepared by an air-drying technique (Thiriot-Quiévreux and Ayraud, 1982) and finally stained with Giemsa 4%, pH 6.8. Ten metaphases from each supposed species were selected, and karyotypes were constructed. Chromosome measurements were then performed with a digitizer tablet (Summa Sketch II) interfaced with a Macintosh computer. Data analysis was performed with an Excel (Microsoft) macro program. Terminology relating to centromere position follows that of Levan *et al.* (1964) and takes into account the confidence limits of the centromeric index means. Nucleolus organizer regions (NORs) were silver-stained directly on unstained slides using the technique of Howell and Black (1982).

## Results

*Mitochondrial DNA sequence analysis*

A PCR fragment of 570 base-pairs from the 16S mitochondrial ribosomal gene was obtained and sequenced for 18 individuals from nine South American and African Atlantic coast samples of mangrove oysters (Table 1). Only two different sequences were obtained; they are denoted by capital letters A and B in Table 1. These were registered in the DDBJ/EMBL/GenBank databases under accession numbers AJ312937 and AJ312938. These two sequences differ in length (473 and 465 bases-pairs) due to 14 insertion or deletion sites. In addition, 45 substitution sites (30 transitions and 15 transversions) were observed. Their divergence, based on Kimura's two-parameter model, was 11.34% (Table 2).

The alignment in Figure 2 is the result of comparing mitochondrial 16S sequences A and B with those obtained for *C. gigas, C. virginica,* and *C. ariakensis* by O'Foighil *et al.* (1995), and those obtained for *S. mordax, S. commercialis* (accession numbers AF353099 and AF353100), and *O. edulis* by Jozefowicz and O'Foighil (1998); *O. edulis*

**Table 2**

*Pairwise sequence divergences, according to Kimura's two-parameter model (Kimura, 1980), among the seven species studied for the 480 nucleotide mt 16S rDNA fragment*

| Species* | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| 1. Sequence A (*C. gasar*) | 0 | | | | | | |
| 2. Sequence B (*C. rhizophorae*) | *0.1132* | 0 | | | | | |
| 3. *C. virginica* | *0.1288* | **0.0357** | 0 | | | | |
| 4. *C. gigas* | *0.1772* | 0.1653 | 0.1657 | 0 | | | |
| 5. *C. ariakensis* | *0.1805* | 0.1923 | 0.1835 | **0.0575** | 0 | | |
| 6. *S. commercialis* | 0.2380 | 0.2185 | 0.2221 | 0.1905 | 0.1758 | 0 | |
| 7. *S. mordax* | 0.2403 | 0.2318 | 0.2187 | 0.1965 | 0.1834 | 0.1021 | 0 |
| 8. *O. edulis* | 0.2314 | 0.2126 | 0.2028 | 0.1823 | 0.1706 | 0.1575 | 0.1716 |

*O. edulis* serves as an outgroup. Two pairwise comparisons yielding low genetic distance estimates are presented in boldface. Species in parentheses are those associated to the sequences described in this report.

* Genera: C., *Crassostrea*; S., *Saccostrea*; O., *Ostrea*.

was considered as an outgroup. The sequence divergences are given in Table 2. Apart from the *O. edulis* outgroup, four groups of sequences can clearly be distinguished on the tree presented in Figure 3: (1) *C. gigas* and *C. ariakensis,* (2) *C. virginica* and sequence B, (3) *S. mordax* and *S. commercialis,* and (4) sequence A. The first three groups are congruent with the three clades of cupped oysters described in O'Foighil and Taylor (2000). Inside the first two groups, divergence is relatively low: 5.75% between *C. gigas* and *C. ariakensis,* and 3.57% between *C. virginica* and sequence B. Inside the *Saccostrea* group, the divergence is higher: 10.21% between *S. mordax* and *S. commercialis*. In this context, sequence A—which displays an 11% divergence with the American *Crassostrea* oysters (group 2), and 17% with the Asian *Crassostrea* oysters (group 1)—can be considered as closer to the American *Crassostrea* oysters. However, whether sequence A is intermediate between the Asian and American *Crassostrea* oysters, or falls within the American *Crassostrea* oysters, is difficult to determine.

### *Geographic distribution of PCR-RFLP haplotypes*

In the PCR-RFLP analysis, using the *Taq*I endonuclease, the two haplotypes (denoted by lower-case letters a and b, corresponding to the sequences A and B, respectively) were rapidly identified. Haplotype b was found only on the South American coast, whereas haplotype a was found on both the African and South American coasts: in French Guiana (SIN) and in two locations in Brazil (PAR1 and CAN) (Table 1, Fig. 1). All locations were monomorphic for one or the other haplotype, but both haplotypes were found within Paranagua Bay among samples PAR1, PAR2, and PAR3.

### *Karyological analysis*

A diploid complement of 20 chromosomes, which is commonly observed in oysters (Nakamura, 1985), was confirmed in the samples. Means of the relative chromosome length and centromeric indexes (Table 3) are given for the individuals from Senegal (sample NOB), presumed to be *C. gasar,* and French Guiana (sample SIN), initially identified as *C. rhizophorae*. The African oyster presented a karyotype (Fig. 4A) of six metacentric and four submetacentric pairs (numbers 2, 8, 9, and 10), while the American (Fig. 4B) showed six metacentric and four submetacentric-subtelocentric pairs (numbers 3, 7, 9, and 10). The position of the large submetacentric pair differed in the African (pair 2) and the American (pair 3) samples, as did a small submetacentric pair (pair 8 in the African samples and pair 7 in the American samples). But taking into account the confidence limits of the relative length means, pairs 2 and 3 of the African oyster may be confounded, as may pairs 7 and 8 (see Table 3). This means that these karyotypes are very similar overall. Furthermore, silver-stained NORs were located on the largest submetacentric chromosome pairs in both samples, and these most probably have the same position in the two karyotypes (Fig. 4, C and D).

## Discussion

The molecular and karyological data reported here extend previous morphological, ecological, and allozyme studies on the taxonomic status and distribution of mangrove oysters from Africa and the east coast of South Africa. Our most striking and important result, however, is that one of the species occurs on both sides of the South Atlantic. This finding compels us to reassess the number of species of South American mangrove oysters, and to consider the mode and direction of the dispersal that must have led to this transoceanic distribution.

In the present study, all the African samples, initially identified as *C. gasar,* were found to be monomorphic for haplotype a, corresponding to sequence A. In contrast,

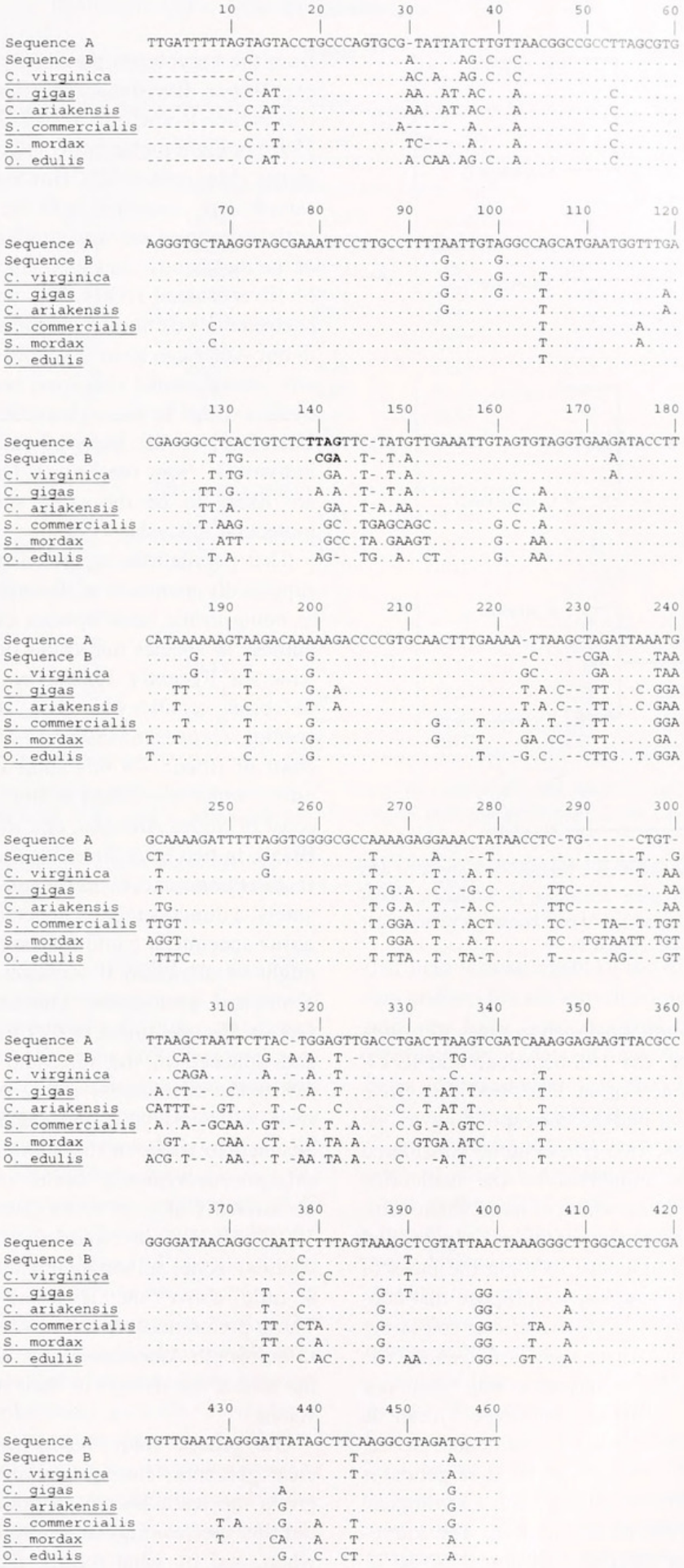


**Figure 2.** Alignment of the sequences A and B, and of published sequences for *Crassostrea virginica, C. gigas,* and *C. ariakensis* (O'Foighil *et al.,* 1995), *Saccostrea commercialis, S. mordax* (Lam and Morton, AF353100), and *Ostrea edulis* (Jozefowicz and O'Foighil, 1998). The four nucleotides in bold at position 141–144 correspond to a polymorphic restriction site for the *Taq*I enzyme.

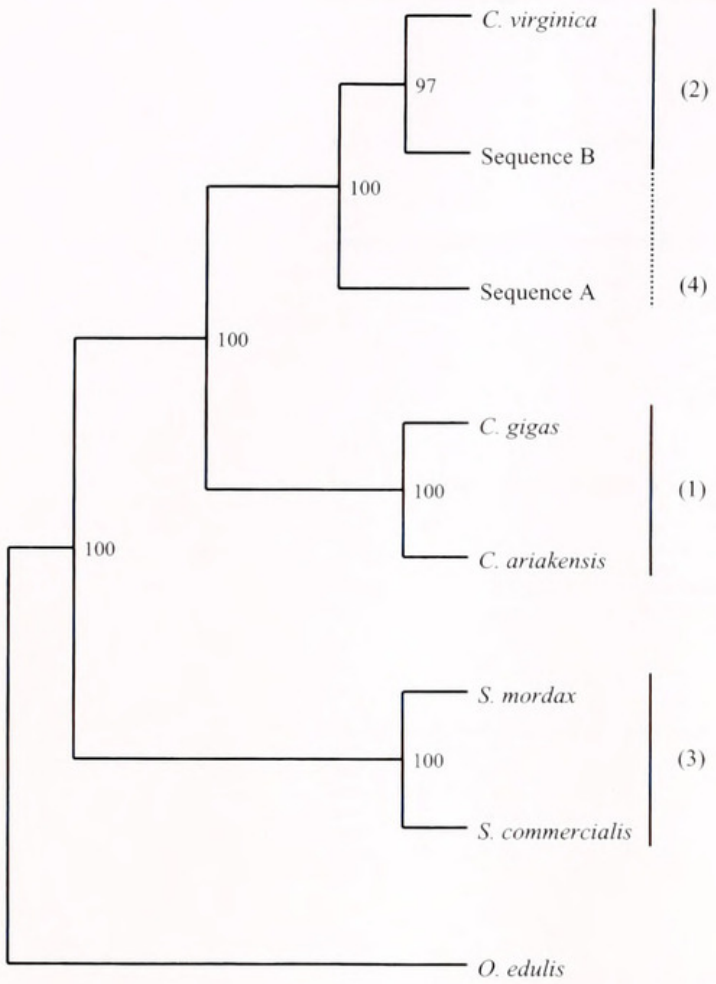


**Figure 3.** A phylogenetic tree based on a parsimony analysis of 480 nucleotide sequences of the 16S gene according to Kimura's model (Kimura, 1980). Numbers on the branches indicate bootstrap values. Four groups of species were identified (1,2,3, and 4).

although almost all of the South American samples were first identified as *C. rhizophorae,* the 16S sequence and RFLP analyses confirm the presence of at least two mangrove oyster species (haplotypes a and b, corresponding to sequences A and B). That these two types can be considered as two different species is supported by the nucleotide divergence (11%) between them, which is large when compared with the divergence calculated between *C. gigas* and *C. ariakensis* on the same fragment (5%). On the basis of our results from the African samples, we propose that haplotype a and sequence A from along American coasts are attributable to the identified African species *C. gasar*. The other species present along the American coasts (sequence B and its corresponding PCR-RFLP haplotype b) can be referred to as *C. rhizophorae* with more confidence. Hence, its close relationship with *C. virginica* (3.5% divergence) is strong evidence of its taxonomic status. The karyological observations support this species distribution. The karyotype of the French Guiana samples (presumed to be *C. rhizophorae*) showed six metacentric and four submetacentric-subtelocentric pairs. This picture is clearly different from the karyotypes previously described for *C. rhizophorae*; that is, five metacentric and five submetacentric pairs (Rodriguez-Romero *et al.,* 1979; Ladron de Guevara *et al.,* 1996) or eight metacentric and two submetacentric chromosomes (Marquez, 1992). However, when the French Guiana samples are compared with the African *C. gasar* samples, both karyotypes are very similar in the number and position of the metacentric and submetacentric pairs and the location of silver-stained NORs. The very slight differences in the centromere position of submetacentric-subtelocentric pairs in our American sample should not be taken as an interspecific chromosomal character, because karyotypes of cupped oysters differ in such characteristics (Leitão *et al.,* 1999). Therefore, on the basis of our karyological observations, individuals from the French Guiana and Senegal samples are likely to be the same species, as was revealed by molecular analysis.

That populations separated by the Atlantic Ocean—and supposedly members of distinct species—are now revealed as being in the same species calls into question the actual number of species that occur on the eastern coast of South America. Recently, Ignacio *et al.* (2000) demonstrated, on the basis of allozyme data, that two distinct biological species, *C. brasiliana* and *C. rhizophorae,* occur along the coast of Brazil. To this short list, our study now adds *C. gasar,* which was found at three locations along the Atlantic coast of South America, one in French Guiana, and two in Brazil, in two bays 70 km apart. Now we must ask whether *C. brasiliana* and *C. gasar* are the same species. Unfortunately, a direct comparison between the *C. brasiliana* and *C. gasar* specimens could not be performed, but the question might be answered if we were to consider the interesting ecological preferences characterized by Ignacio *et al.* (2000). His specimens of *C. rhizophorae* oysters were small and attached in the intertidal zone, either to mangrove (*Rhizophorae mangle*) roots, or to rocks in the intertidal zone. Conversely, *C. brasiliana* oysters were larger and attached to rocks in the subtidal zone. In comparison, *C. rhizophorae* typically settles on the mangrove roots, but occasionally also on rocks (Nascimento *et al.,* 1991; Rios, 1994). Finally, in Nigeria (Africa), *C. gasar* favors the subtidal zone, although it can, in the dry season, occur a little above the level of low tide (Ajana, 1980). These preferences do not resolve species relationships, and in our South American study, we could not correlate either the size of the oysters or their habitat with their taxonomic status.

Our genetic study, based on the mitochondrial and nuclear genomes, clearly demonstrates that a common mangrove species is present in South America and Africa. But did this species originate in South America or Africa? And when, and by what means, did the dispersal occur? The identical mtDNA sequences of American and African *C. gasar* oysters show that these two population groups have a

**Table 3**

*Chromosome measurements derived from 10 cells of each sample*

| Chrom. pair no. | Mean RL | Conf. L. | RL max | RL min | Mean CI | Conf. L. | CI max | CI min | Type* |
|---|---|---|---|---|---|---|---|---|---|
| | | | | NOB sample: putative *Crassostrea gasar* | | | | | |
| 1 | 11.36 | 0.48 | 11.84 | 10.89 | 43.64 | 2.34 | 45.99 | 41.30 | m |
| 2 | 11.19 | 0.53 | **11.72** | **10.67** | 27.52 | 1.67 | 29.19 | 25.84 | sm |
| 3 | 11.04 | 0.29 | **11.33** | **10.75** | 45.62 | 1.77 | 47.39 | 43.85 | m |
| 4 | 10.62 | 0.54 | 11.16 | 10.08 | 37.80 | 1.65 | 39.45 | 36.14 | m/sm |
| 5 | 10.54 | 0.36 | 10.90 | 10.18 | 46.95 | 1.75 | 48.70 | 45.20 | m |
| 6 | 9.97 | 0.31 | 10.28 | 9.66 | 45.77 | 1.66 | 47.43 | 44.10 | m |
| 7 | 9.64 | 0.39 | **10.03** | **9.25** | 45.21 | 2.53 | 47.75 | 42.68 | m |
| 8 | 9.54 | 0.45 | **9.99** | **9.08** | 30.78 | 2.45 | 33.23 | 28.33 | sm |
| 9 | 8.82 | 0.43 | 9.25 | 8.39 | 28.65 | 2.15 | 30.80 | 26.49 | sm |
| 10 | 7.28 | 0.40 | 7.68 | 6.88 | 27.89 | 2.70 | 30.59 | 25.19 | sm |
| | | | | SIN sample: putative *Crassostrea rhizophorae* | | | | | |
| 1 | 12.08 | 0.40 | 12.48 | 11.68 | 44.51 | 1.76 | 46.26 | 42.75 | m |
| 2 | 11.83 | 0.48 | 12.31 | 11.35 | 42.57 | 2.52 | 45.09 | 40.05 | m |
| 3 | 10.86 | 0.36 | 11.22 | 10.50 | 26.02 | 2.37 | 28.39 | 23.66 | sm/st |
| 4 | 10.73 | 0.41 | 11.15 | 10.32 | 44.15 | 2.95 | 47.10 | 41.21 | m |
| 5 | 10.58 | 0.60 | 11.18 | 9.98 | 37.00 | 2.22 | 39.22 | 34.78 | m/sm |
| 6 | 9.87 | 0.29 | 10.16 | 9.58 | 42.65 | 2.16 | 44.81 | 40.50 | m |
| 7 | 9.76 | 0.42 | **10.18** | **9.33** | 24.98 | 1.35 | 26.33 | 23.63 | sm/st |
| 8 | 9.15 | 0.56 | **9.71** | **8.59** | 43.57 | 1.39 | 44.96 | 42.19 | m |
| 9 | 8.76 | 0.27 | 9.04 | 8.49 | 24.29 | 1.00 | 25.29 | 23.29 | sm/st |
| 10 | 6.37 | 0.53 | 6.90 | 5.84 | 26.07 | 2.23 | 28.30 | 23.85 | sm/st |

CI: centromeric index, Conf. L.: confidence limit of means, RL: relative length, m: metacentric, sm: submetacentric, st: subtelocentric. The boldface values correspond to the confidence limits of the relative length means of chromosome pairs that allow pairs 2 and 3 to be confounded, and likewise pairs 7 and 8.

* The morphological type of chromosome is given according to the CI max and CI min; m/sm means that the type of the chromosome pair is on the edge of the two classes—metacentric and submetacentric (see classification in Levan *et al.*, 1964).

relatively recent common origin. O'Foighil *et al.* (1998) analyzed sequences of the cytochrome oxidase I gene and dated the genetic divergence between *C. gigas* and *C. angulata* at 1 to 2 million years. When the 16S ribosomal gene was analyzed, the divergence between the two taxa was estimated to be 0.46% (Huvet, 2000; unpubl. data provided by O'Foighil). If we assume that mutation rates at the cytochrome oxidase I gene are similar among *Crassostrea* species, then the African and American populations of *C. gasar* were established less than 1 to 2 million years ago. To assess the level of polymorphism of the 16S ribosomal gene in *C. gasar,* a larger sample size would be necessary. For example, Small and Chapman (1997) used a PCR-RFLP approach (10 restriction enzymes) on 410 individuals from the Atlantic coasts and the Gulf of Mexico to study intraspecific variation in the 16S ribosomal gene of *C. virginica.* They found 11 haplotypes, of which one was highly frequent (95 %). Thus, the level of genetic divergence between the American and African *C. gasar* populations could probably be better estimated if more polymorphic markers were available.

The relatively recent divergence between the American and African populations of *C. gasar* supports the hypothesis that man was an agent of the dispersal. Many recent transfers and worldwide introductions of oysters for aquacultural purposes have been reported (Carlton and Mann, 1996). Furthermore, events of introduction in historic time have been demonstrated on the basis of genetic markers (O'Foighil *et al.,* 1998; Boudry *et al.,* 1998). In the case of *C. gasar,* shipping between South America and the west coast of Africa may have transferred these oysters from one coast to the other. Note, however, that no *C. rhizophorae* samples were found in Senegal or Nigeria. Although the west African coast must be more extensively sampled to confirm this result, it does imply that, unlike *C. gasar, C. rhizophorae* either was not transported to or did not persist on the African coast.

Natural transport may also be responsible for the present geographic range of *C. gasar.* Larval dispersal might have been possible, even over such a long distance (at least 3000 km), because the larval stage of most *Crassostrea* species lasts about 3 weeks. However, the transport of adult oysters on drifting objects, a common phenomenon in the marine environment, may be a mode of dispersal with a larger potential range than that achievable by swimming larvae (Johannesson, 1988; O'Foighil, 1989). For example, dis-


**Figure 4.** (A) Giemsa-stained karyotype of African *Crassostrea gasar,* (B) Giemsa-stained karyotype of putative *C. rhizophorae* from French Guiana, (C) NOR-stained karyotype of African *C. gasar,* (D) NOR-stained karyotype of putative *C. rhizophorae* from French Guiana. Scale bar = 5 μm.

persal by rafting was the most likely explanation for trans-Pacific range extension by the flat oyster *Ostrea chilensis* from New Zealand to Chile (O'Foighil *et al.,* 1999). The predominant surface circulation patterns in this part of the Atlantic Ocean (Fig. 1) favor the hypothesis that *C. gasar* was transported from Africa to America, as also hypothesized by Lessios *et al.* (1999) to explain the close genetic similarity of specimens of *Eucidaris tribuloides* from the Caribbean Sea and Brazil to those from the Gulf of Guinea.

Focusing on the Paraguana Bay (Brazil) where two species were found, PAR1 exhibited the *C. gasar* haplotype, but PAR2 and PAR3, both located in the same bay about 30 km away, exhibited the *C. rhizophorae* haplotype. One can ask whether these species are completely or incompletely reproductively isolated, and whether they have different habitats. A more intensive survey could provide an answer by revealing whether individuals from these species inhabit the same site, and whether hybrids occur in the wild. Based on the rRNA large subunit DNA sequences, and those known between other species in the genus (see Table 2), the genetic distance between *C. gasar* and *C. rhizophorae* is sufficiently large (88.7% similarity) that they are unlikely to produce viable hybrids. Indeed, the genetic distance between *C. gigas* and *C. virginica,* two species for which viable hybrids could not be obtained (Allen *et al.,* 1993), is of similar value (84.5% similarity).

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