



Phylogeography of the copepod *Calanoides carinatus* s.l. (Krøyer) reveals cryptic species and delimits *C. carinatus* s.s. distribution in SW Atlantic Ocean



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ABSTRACT

Calanoides carinatus sensu lato (s.l.) (Krøyer, 1848) is known to occur in the North and South Atlantic, Indian, and tropical western Pacific Oceans, and the Mediterranean Sea. In highly productive areas, this species may dominate the copepod biomass and occupy a pivotal position in the pelagic food webs that support commercial fisheries. Despite the species' ecological importance, its taxonomic status and biogeographic range are not certain. An international collaborative project was carried out to examine the molecular phylogeography and population genetics of *C. carinatus* of the Atlantic Ocean. A total of 55 specimens were identified from 13 samples collected from neritic populations of the SW (23°S–47°S), SE (16°S–34°S) and NE (11°N–44°N) Atlantic Ocean. A 708 base-pair (bp) region of the mitochondrial cytochrome *c* oxidase subunit I (mtCOI) gene was amplified. DNA sequences were trimmed to a final aligned length of 484 bp for analysis. A parsimony haplotype network (constructed with TCS Ver. 1.2.1) had no shared haplotypes between the SE and SW Atlantic samples. In contrast, SE and NE Atlantic samples had broadly shared haplotypes, although several individuals from the NE Atlantic had very divergent haplotypes. Based on all samples analyzed, neutrality tests were both positive and significant (Tajima's $D = 2.374$, $p < 0.05$; Fu's $FS = 29.378$, $p < 0.001$) and haplotype diversity (H_d) was 0.81. NE and SE Atlantic samples were pooled for analysis (based on average pairwise Tamura-Nei distance between *C. carinatus* s.l. individuals = 0.024). Between SW and NE/SE Atlantic populations, average pairwise distance = 0.556 and $\varphi_{ST} = 0.956$ ($p < 0.0001$). Phylogeographic analysis of mtCOI sequence variation indicates that *C. carinatus* s.l. comprises two genetically divergent and geographically distinct species. Since the type locality of the species is in Brazilian waters, we consider the SW Atlantic type to represent *C. carinatus sensu stricto* (s.s.), with a biogeographical range restricted to the SW Atlantic Ocean. Further, we consider the NE/SE Atlantic type to be an undescribed, cryptic sibling species.

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1. Introduction

1.1. Historical taxonomy of *Calanoides carinatus* s.l.

In the late summer of 1840, the prominent Danish zoologist Henrik Krøyer accompanied the Danish navy frigate *Bellona* to South America (Damkaer and Damkaer, 1979). The cruise provided an excellent opportunity to make important new collections from coastal waters near Madeira, Brazil, Chile, and also from the open ocean. Among these collections was a single adult female of a new species described as "*Calanus carinatus*" (Krøyer, 1848). The specimen was delivered to the systematic crustacean collection of the Danish Royal Museum of Natural History (Damkaer and Damkaer, 1979). The

description was very short and no drawings of the new species were provided. The only mention concerning the type locality in the original publication of Krøyer was “I have found a species of genus *Calanus* in the Atlantic Ocean at 30° south, close to the coast of Brazil” (Damkaer and Damkaer, 1979). Later, the genus was reassigned to *Calanoides* (Brady, 1883) by Scott (1909). Subsequent taxonomic analysis has indicated that there may be more than one species within *C. carinatus* (Bradford, 1988; Peterson, 1998; Verheye et al., 2005), and the species is now typically referred to as *C. carinatus sensu lato* (s.l.).

1.2. Biogeographical distribution and ecological role of *C. carinatus* s.l.

C. carinatus sensu lato (s.l.) (Krøyer, 1848) is distributed throughout tropical and subtropical areas of the N and S Atlantic and Indo-Pacific Oceans, as well as in the Mediterranean Sea (Razouls et al., 2005, 2014). It is a characteristic and dominant species in upwelling systems (Valentin et al., 1987; Verheye et al., 1992), where it is thought to play a crucial role in energy flux through the pelagic food web (Verheye et al., 2005).

In the NE Atlantic Ocean, *C. carinatus* s.l. has been found in deep oceanic waters southwest of the British Isles (Williams and Conway, 1988); off the coast of Portugal (John et al., 1998; Williams and Conway, 1988) and Spain (Ceballos and Álvarez-Marqués, 2006; Ceballos et al., 2006). The species has been reported in upwelling areas off NW Spain (Ceballos et al., 2004) and Africa (Schnack-Schiel, 1982; Schulz, 1982); and off equatorial West Africa, including the Ivory Coast (Binet and Suisse de Sainte Clair, 1975), Ghana (Mensah, 1974), and the Congo (Petit and Courties, 1976). It occurs off the coasts of Angola (De Decker, 1984 and references therein), Namibia (Timonin et al., 1992; Unterüberbacher, 1964), and South Africa (De Decker, 1964; Verheye et al., 1992). In the northern and southern Benguela upwelling regions (Timonin et al., 1992; Verheye et al., 1992 and references therein), the species can dominate the copepod community in abundance (up to 36%) and biomass (up to 67%; Verheye, 1991).

In the Indian Ocean, the species has been found off Somalia, in the Gulf of Aden (Baars et al., 1998; Smith, 1984). De Decker (1984) regularly found *C. carinatus* s.l. in the south of Madagascar, along the south and east coast of Australia, and in the Indo-Malayan area. The species has been also reported in the upwelling system of Java (Tranter, 1977), in the Philippine Sea, the South and East China Sea and the Izu region off Japan (Razouls et al., 2005, 2014; Unterüberbacher, 1964 and references therein).

In the SW Atlantic, *C. carinatus* s.l. is distributed in Brazilian neritic waters from 20° to 30°S (Campaner and Honda, 1987 and references therein; Valentin and Monteiro Ribas, 1993). The species has been reported as an indicator species of upwelling waters off Cape Frío, Brazil (Valentin and Monteiro Ribas, 1993; Valentin et al., 1987) and has been also recorded off Rio Grande do Sul, specifically in the surf zone of Tramandaí Beach (Ávila et al., 2009) and Cassino Beach (Bersano, unpubl.). In the Argentine Sea, *C. carinatus* s.l. is distributed from 34°S to 47°S in coastal and mid-shelf waters (Marrari et al., 2004; Ramírez, 1981; Ramírez and Sabatini, 2000; Viñas et al., 2002), specially associated with productive frontal regions (Sabatini and Martos, 2002; Santos and Ramírez, 1995; Viñas et al., 2002).

C. carinatus s.l. occupies a pivotal position in fisheries food webs. The species constitutes a dominant food item for important pelagic fishes, such as the chub mackerel, *Scomber japonicus* (Perrotta et al., 1999; Viñas et al., 1999), the Brazilian sardine, *Sardinella brasiliensis* and the Argentine anchovy, *Engraulis anchoita* (Campaner and Honda, 1987; Schwingel and Castello, 1994) off Argentina and Brazil, as well as the European anchovy, *Engraulis encrasicolus*, the Pacific sardine, *Sardinops sagax*, the round herring, *Etrumeus whiteheadi*, and many other fishes off South Africa (Armstrong et al., 1991).

1.3. More than one species within *Calanoides carinatus* s.l.?

Variation in morphological characters (e.g., male fifth legs) and/or differences in life cycle between *C. carinatus* s.l. from diverse regions suggests the existence of more than one species (Bradford, 1988; Peterson, 1998; Sabatini et al., 2007; Verheye et al., 2005).

Sabatini et al. (2007) analyzed the historical taxonomy of *C. carinatus* s.l. in detail and produced a precise morphological redescription of *C. carinatus sensu stricto* (s.s.) based on collections from a location near the species' putative type locality. In addition, female and male specimens collected off Brazil and Argentina were compared morphologically and no significant differences were found. Sabatini et al. (2007) therefore concluded that specimens collected from the various South American locations were conspecific, although they suggested genetic confirmation of this conclusion.

On the other hand, in a study of life cycle of *C. carinatus* s.l. in the Angola and Benguela Currents in the SE Atlantic, the observation of morphologically different individuals in different parts and/or depth strata along the coast led Verheye et al. (2005) to suspect the existence of genetical differences between populations.

1.4. Phylogeographic analysis

Patterns of genetic diversity within and between species may be used to reconstruct the evolutionary relationships among species, assess the significance of geographic variation, reveal cryptic species, and accurately estimate biodiversity. For zooplankton in particular, population genetic analysis can be used to infer how dispersal may be influenced by transport in ocean currents (Bucklin, 2000; Goetze, 2003; McManus and Katz, 2009). Phylogeographic analysis explicitly considers the spatial distribution of genetic diversity and structure within and between species to allow quantitative estimates of dispersal (gene flow), as well as examination of the impacts of demographic history and evolutionary events (Avice, 2000).

Mitochondrial cytochrome *c* oxidase I (mtCOI) has been used as a genetic marker for discrimination and identification of species for a wide variety of marine organisms (Bucklin et al., 2011). Variation of mtCOI within a species is far less than variation between species for most taxa studied, making the gene a diagnostic molecular systematic character of even closely-related and cryptic species (Meyer and Paulay, 2005). For calanoid copepods in particular, previous studies have demonstrated the usefulness of mtCOI sequence variation for accurate and reliable discrimination of species – even very closely-related and sibling species (e.g., Blanco-Bercial et al., 2014; Bucklin et al., 2003, 2010a) – and for phylogeographic analysis of wide-spread species (e.g., Blanco-Bercial et al., 2011).

The present study summarizes the results from an international collaboration designed to examine the population genetics and phylogeography of *C. carinatus* s.l. in large sectors of the SW (23°S–47°S), SE (16°S–34°S) and NE (11°N–44°N) Atlantic Ocean. Our primary objective is to examine persistent questions of the taxonomy and biogeography of *C. carinatus* s.l. across a large portion of its putative geographic range. The genetic marker chosen is a portion of the mitochondrial cytochrome *c* oxidase I (mtCOI) gene, called the barcode region (Hebert et al., 2003), which has been shown to discriminate closely-related and cryptic species of copepods (e.g., Blanco-Bercial et al., 2014; Bucklin et al., 2003, 2010a,b).

2. Material and methods

2.1. Collection and preservation of samples

Samples were collected from continental and oceanic waters in the SW Atlantic off the coasts of Brazil (23°S–33°S, including the type locality) and Argentina (38°S–47°S); SE Atlantic, off the southwest coast of

Africa (16°S–34°S); and NE Atlantic, off the coasts of Western Sahara, Guinea, and Spain (32°N–43°N) (Table 1, Fig. 1). Samples were preserved in 95% undenatured ethyl alcohol (EtOH) for molecular analysis using the protocol from Bucklin (2000). Females or fifth copepodite (CV) stages of *C. carinatus* s.l. were identified and specimens selected for analysis were placed in vials with EtOH. Samples were shipped to the University of Connecticut (UConn) for molecular analysis.

2.2. Specimen and DNA vouchers

Specimen vouchers were archived at UConn in 95% ethyl alcohol and stored at –20 °C. DNA vouchers were retained and stored in freezers at UConn. Data and metadata associated with each specimen analyzed were included in a specimen-tracking database created using Microsoft

ACCESS software. Additional formalin-preserved specimens corresponding to each of the sampling locations were archived at the home institutions of the collaborating researchers.

2.3. Molecular analysis

DNA extraction was performed with the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) using standard protocols, except that elution volumes varied based on individual size (usually 100–200 µL). A 708 base-pair (bp) region of mtCOI was amplified in a GeneAmp 9600 PCR machine (Applied Biosystems, Inc., Carlsbad, CA) using consensus PCR primers (Folmer et al., 1994) and published amplification protocols (Bucklin et al., 2010b). PCR products were cleaned with QIAquick PCR Purification Kit (Qiagen, Valencia, CA). DNA sequences

Table 1

Sampling localities including countries, geographic position, collection dates, researchers who identified specimens, numbers of individuals analyzed, sequence identification and GenBank Accession Numbers.

Sampling localities	Latitude/longitude	Collection date	Identified by	Number of individuals	Sequence ID	GenBankAcc No.
1. Cape Frío, Brazil	23.00 S 42.00 W	27-Jan-2005	W. Monteiro-Ribas	2	CalCF1.3	KC287487
					CalCF1.4	KC287488
2. Cassino Beach, Brazil	32.20 S 52.17 W	29-Dec-2004	J.G.F. Bersano	5	JS61.1	KC287435
					JS60.4	KC287449
					JS61.2	KC287450
					JS61.3	KC287451
					JS61.4	KC287452
3. Miramar, Argentina	38.47 S 57.68 W	12-Jun-2003	M.D. Viñas	2	EPEAJS376	KC287485
					EPEAJS377	KC287486
4. Escondida Island, Argentina	43.82 S 64.23 W	29-Jan-2004	M.D. Viñas	3	KS63.5	KC287433
					KS63.6	KC287434
5. San Jorge Gulf, Argentina	46.75 S 66.27 W	27-Oct-2005	M.D. Viñas	6	KS63.4	KC287436
					CalSJG1.1	KC287462
					CalSJG1.2	KC287463
					CalSJG1.3	KC287464
					CalSJG1.6	KC287465
6. Bay of Biscay, Spain	43.70 N 6.15 W	2004	S. Ceballos	12	CalSJG1.7	KC287466
					CalSJG1.8	KC287467
					CcarBB1	KC287437
					CcarBB10	KC287438
					CcarBB11	KC287439
					CcarBB12	KC287440
					CcarBB2	KC287441
					CcarBB3	KC287442
					CcarBB4	KC287443
					CcarBB5	KC287444
					CcarBB6	KC287445
					CcarBB7	KC287446
7. Gulf of Cadiz, Spain	36.29 N 6.62 W	3-May-2007	L. Blanco-Bercial	7	CcarBB8	KC287447
					CcarBB9	KC287448
					CalSpa41	KC287468
					CalSpa51	KC287469
					CalSpa61	KC287470
					CalSpa62	KC287471
					CalSpa91	KC287473
					CalSpa93	KC287472
8. Western Sahara	24.68 N 20.75 W	5-Nov-2007	J. Bradford Grieve	5	CalSpa92	KC287474
					Co140.6.2	KC287476
					Co140.6.3	KC287477
					Co140.6.4	KC287478
					Co140.6.5	KC287479
					Co140.6.6	KC287480
9. Guinea	11.68 N 20.42 W	8-Nov-2007	L. Blanco-Bercial	4	Co140.7.1	KC287481
					Co140.7.2	KC287482
					Co140.7.3	KC287483
					Co140.7.4	KC287484
					CalSAfr71	KC287460
10. Angola	16.33 S 11.00 E	3-Feb-2004	H. Verheye	2	CalSAfr72	KC287461
					CalSAfr81	KC287455
11. Namibia	26.10 S 13.23 E	18-Apr-2004	H. Verheye	3	CalSAfr11	KC287456
					CalSAfr12	KC287457
12. Lüderitz, Namibia	28.95 S 14.25 E	22-Apr-2004	H. Verheye	2	CalSAfr131	KC287453
					CalSAfr132	KC287454
13. Cape Peninsula, South Africa	33.88 S 18.40 E	11-Jan-2005	H. Verheye	2	CalSAfr31	KC287458
					CalSAfr32	KC287459

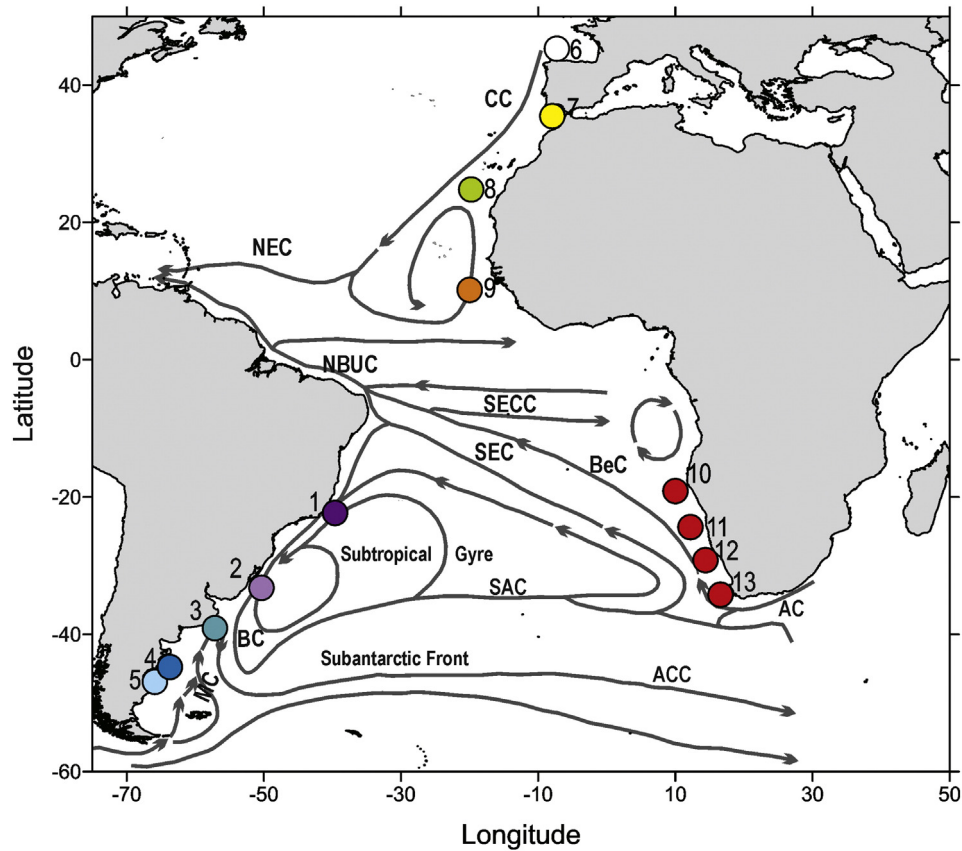


Fig. 1. Sampling locations of *Calanoides carinatus* s.l. for molecular analysis in the Atlantic Ocean, with diagrammatic representation of oceanographic currents as described in the text (modified from Peterson and Stramma, 1991). Abbreviations are: AC: Agulhas Current; ACC: Antarctic Circumpolar Current; BC: Brazil Current; CC: Canary Current; MC: Malvinas (= Falkland) Current; NBUC: North Brazil Undercurrent; NEC: North Equatorial Current; SAC: South Atlantic Current; SEC: South Equatorial Current; SECC: South Equatorial Counter-current. Numbers refer to sampling localities (see also Table 1). Color of the circles indicates the source locations for the TCS analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

were determined directly from PCR amplification products using the forward and reverse primers. The sequencing reactions were carried out using the BigDye Terminator Ver. 3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) at 1/4 standard volume in an ABI GeneAmp 9600 PCR machine. DNA templates were purified and suspended in ABI Hi-Di Formamide for sequencing.

DNA sequencing was done using an ABI 3130 Genetic Analyzer, with an array of four 50-cm capillaries, which was operated using standard conditions. A 1-hour electrophoresis run time produced approximately 500–700 base-pair (bp) reads in one direction, providing complete or almost complete bi-directional coverage of the target mtCOI region. All sequences were manually checked for accurate machine reading using the Molecular Evolutionary Genetics Analysis (MEGA, Ver. 4) software package (Tamura et al., 2007) and the DNA sequence assembly program, Sequencher Ver. 4.10.1 (Gene Codes Corp., Ann Arbor, MI).

The mtCOI sequences were aligned using CLUSTAL-W, as implemented in MEGA (Thompson et al., 1997). The complete alignment was trimmed to a length of 650 bp for preliminary analysis to confirm the accuracy and validity of the sequences. DNA sequences that could not be verified and validated, including aberrant or highly divergent sequences, were omitted from the dataset. The designated GenBank Accession Numbers (Table 1) can be used to access the GenBank record, which includes data and metadata for each specimen.

2.4. Analysis of genetic diversity and structure

A variety of tests of levels and patterns of genetic variation were carried out, both to ensure the suitability of the genetic markers for

population genetic analysis and to examine genetic diversity and structure of *C. carinatus* s.l. The degree of substitution saturation was examined prior to analysis, using the test of Xia et al. (2003) implemented in DAMBE (Xia and Lemey, 2009), in order to detect sequences with substantial levels of saturation, which would make them unsuitable for analysis. A parsimony haplotype network was constructed with TCS Ver. 1.2.1 (Clement et al., 2000), in order to visualize the diversity and phylogenetic relationships among the different haplotypes and provide qualitative assessment of their geographic distributions. Neutrality was tested with Tajima's *D* (Tajima, 1989) and Fu's *F_S* (Fu, 1997) using DnaSP Ver. 5 (Librado and Rozas, 2009); the same software was used to calculate the haplotype diversity (*H_d*) for each population. The best-fitting substitution model was determined with jModelTest (Posada, 2008). Once the appropriate nucleotide substitution model was selected, the estimated parameters and most similar available model were used to calculate the values of genetic differentiation among populations, φ_{ST} in Arlequin Ver. 3.5.1.2 (Excoffier and Lischer, 2010). The φ_{ST} is based upon both the haplotype frequencies, used to calculate the original *F_{ST}* (Wright, 1949), and also the evolutionary distances among different haplotypes. The same model was implemented in MEGA Ver. 4 (Tamura et al., 2011) in order to calculate pairwise genetic distances between populations of *C. carinatus* s.l. and also between *C. carinatus* s.l. and the congeneric species, *Calanoides macrocarinatus* (GenBank Acc. No. AF332790) and *Calanoides acutus* (GenBank Acc. No. AF332791), using *Mesocalanus tenuicornis* (GenBank Acc. No. AF332788) as the outgroup. Pairwise differences (i.e., average number of nucleotide substitutions) were calculated between individuals of each sample, between regional populations of *C. carinatus* s.l., and between the species listed above.

3. Results

A total of 55 individuals of *C. carinatus s.l.* collected at 13 sampling locations was analyzed (Table 1). The aligned length of the sequences used for the definitive analyses was 484 bp. The test of substitution in DAMBE indicated low levels of saturation for the COI sequence; the data were thus deemed suitable for phylogenetic analysis.

The TCS network diagram indicated complete isolation between the SW and NE/SE Atlantic populations, with no shared haplotypes between them (Fig. 2). In addition, TCS analysis indicated no differentiation between NE and SE populations, with two groups of haplotypes found in both regions. Several individuals from the NE Atlantic, at the northern edge of the species distribution, showed very divergent haplotypes (Fig. 2). Despite the differences, all the sequences coded for the same amino acid sequence.

Both neutrality tests were positive and significant based on all samples analyzed (Tajima's $D = 2.374$, $p < 0.05$; Fu's $F_S = 29.378$, $p < 0.001$); separate analysis of the SW Atlantic population yielded $D = -0.438$ n.s. and $F_S = -0.377$ n.s.; for NE/SE samples, $D = -0.780$ n.s. and $F_S = 5.631$ ($p < 0.01$). Haplotype diversity (H_d) based on all sampled populations was 0.81. The SW Atlantic population had $H_d = 0.451$, while the $H_d = 0.751$ for the NE/SE Atlantic population. The nucleotide substitution model chosen by jModelTest was the Tamura and Nei model (Tamura and Nei, 1993), with a gamma distribution defined by $\alpha = 0.225$. Once implemented in

Arlequin, the genetic distance obtained between the NE/SE and SW Atlantic populations was $\varphi_{ST} = 0.956$ ($p < 0.0001$).

Pairwise Tamura-Nei genetic distances between *C. carinatus s.l.* and other species ranged from 0.615 to 0.804. The average Tamura-Nei distance between *C. carinatus s.l.* individuals of NE and SE populations was 0.024, which justified pooling these samples for the subsequent analysis. The average pairwise distance between NE/SE and SW Atlantic populations was 0.556 (Table 2). Between populations within either region, the average nucleotide difference was 0.01% (range 0.0–0.5%) in the SW Atlantic and 2.3% (range 0.0–9.9%) in the NE/SE Atlantic. Excluding the two highly divergent haplotypes from the NE Atlantic samples, average nucleotide difference between populations in the NE/SE Atlantic was 0.6% (range 0.0–1.4%).

4. Discussion

4.1. Genetic divergence of *C. carinatus s.l.* of NE/SE and SW Atlantic

Molecular phylogeographic analysis of samples collected from selected coastal areas of the NE, SE and SW Atlantic indicated that *C. carinatus s.l.* comprises two geographically distinct and genetically divergent populations, one found in coastal regions of the SW Atlantic off Brazil and Argentina (20°S to 47°S), and a second genetically-cohesive population distributed from the NE Atlantic (Bay of Biscay) to the SE Atlantic (off the coast of Africa). The mtCOI gene region sequenced differed by an average of 21% between the NE/SE and SW Atlantic populations, which is consistent with differences between distinct species of calanoid copepods (Blanco-Bercial et al., 2014) and other marine invertebrates (Bucklin et al., 2011). All statistical analyses supported the genetic distinction of NE/SE and SW Atlantic populations, including analysis of population genetic structure ($\varphi_{ST} = 0.956$; $p < 0.0001$) and measures of genetic distance (average Tamura-Nei distance = 0.556). Considering the low number of individuals analyzed from the western Atlantic, the power of the haplotype diversity analysis is too low to infer adequate conclusions, and therefore these results are not discussed. The magnitude of the differences based in the other analyses (TCS, genetic distances) made them very robust against the limited sampling.

Since the type locality of the species is near the Brazilian collections, and since recent morphological taxonomic analysis of specimens of *C. carinatus* from this region by Sabatini et al. (2007) revealed no differences from the original species description, we conclude that the SW Atlantic populations represent *C. carinatus s.s.* and that the species' geographical distribution is most likely restricted to the SW Atlantic Ocean.

The reproductive isolation and genetic divergence of populations of *C. carinatus s.l.* from coastal regions of the SW and SE Atlantic Ocean are not easily explained by the present-day circulation patterns of the major oceanographic currents in the South Atlantic. Major current systems connect the margins of the ocean both on the surface (Peterson and Stramma, 1991) and in deeper levels of the water column (Stramma and England, 1999). Close to South Africa, the Benguela Current (BeC) flows northwest around the Subtropical Gyre, and then feeds the South Equatorial Current (SEC) (Fig. 1). The SEC transports near-surface water onto the Brazilian shelf near 16°S, and then separates into the northward-flowing North Brazil Undercurrent (NBUC) and the southward-flowing Brazil Current (BC), with the latter mixing with the Malvinas (Falkland) Current (MC) and leaving the shelf to flow eastward as the South Atlantic Current (SAC). Near South Africa, a branch of the SAC flows eastward to the Indian Ocean, while the other turns North into the BeC and finally into the SEC.

Although it is not possible to accurately estimate the likelihood and timing of transport through this series of currents, it seems possible – if not likely – that isolation by distance and limited advective transport may allow genetic divergence between copepod populations in the NE/SE versus SW Atlantic regions. Active individuals of *C. carinatus s.l.*

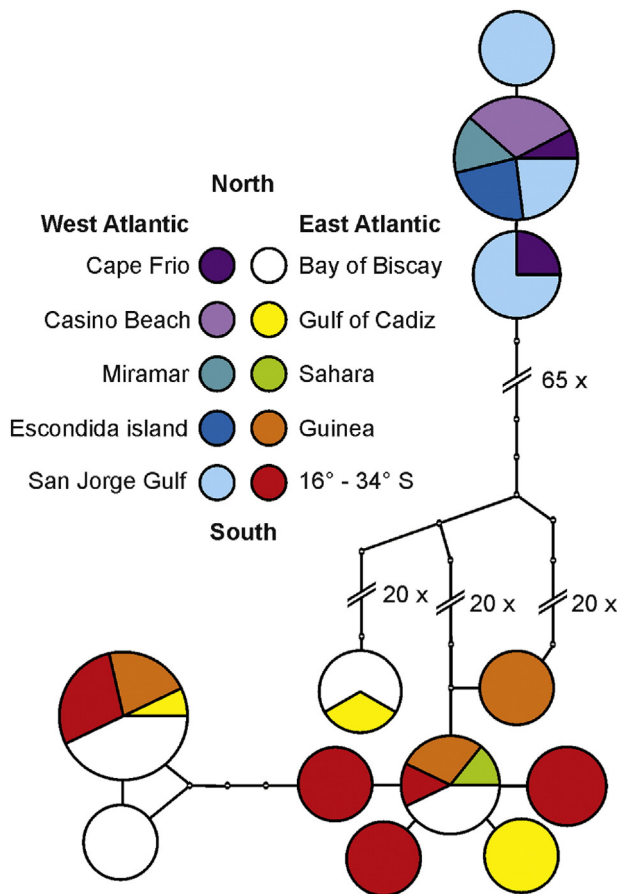


Fig. 2. TCS Network diagram showing frequencies, relationships and source locations for the 12 haplotypes observed for *Calanoides carinatus s.l.* The size of the pie indicates relative frequency; color of the slice indicates the collection area (see also Fig. 1); samples in legend are ordered from North to South. Due to the low number of individuals, the SE Atlantic samples from 16° to 34°S (Benguela Current) were pooled. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2

Matrix of Tamura-Nei genetic distances based on mtCOL sequences (above diagonal) and percentage of nucleotide differences (below diagonal) between *Calanoides carinatus* s.l. of the SW and NE/SE Atlantic, *C. macrocarinatus*, *C. acutus* and *Mesocalanus tenuicornis*. See text for details of calculations.

	<i>C. carinatus</i> –SW Atlantic	<i>C. carinatus</i> –NE/SE Atlantic	<i>C. acutus</i>	<i>C. macrocarinatus</i>	<i>M. tenuicornis</i>
<i>C. carinatus</i> –SW Atlantic		0.556	0.615	0.804	0.662
<i>C. carinatus</i> –NE/SE Atlantic	(19.8%)		0.722	0.771	0.733
<i>C. acutus</i>	(20.7%)	(20.4%)		0.622	1.000
<i>C. macrocarinatus</i>	(21.3%)	(20.6%)	(20.5%)		0.774
<i>M. tenuicornis</i>	(21.0%)	(21.0%)	(24.0%)	(21.3%)	

would not be expected to survive transport across the South Atlantic Ocean, because of food limitation in the oligotrophic central gyre (Moreno-Ostos et al., 2011). Indeed, Verheye et al. (2005) found that active, surface-dwelling juveniles (copepodite stage CV) and adults of *C. carinatus* s.l. in the SE Atlantic required a regular food supply, and that their limited lipid reserves would not sustain their survival for more than 10 days. The maximum survival time of diapausing CV copepodites of *C. carinatus* s.l. was estimated to be 149–192 days (Verheye et al., 2005), suggesting that it is unlikely that diapausing individuals could be transported alive across the basin.

4.2. Genetic divergence of *C. carinatus* s.s. and s.l. from *C. macrocarinatus* and *C. acutus*

C. carinatus s.s. (SW Atlantic) and *C. carinatus* s.l. (NE/SE Atlantic) are clearly genetically differentiated from both *C. macrocarinatus* and *C. acutus* (Table 2). Accordingly, Sabatini et al. (2007) found morphological differences between specimens of *C. carinatus* s.s. and *C. macrocarinatus* from New Zealand and concluded that they were different species. They also found that both species may be distinguished from *C. acutus* by several morphological characters of the female and male.

4.3. Phylogeography and ecology of *C. carinatus* s.l. in the NE and SE Atlantic

Phylogeographic analysis of samples of *C. carinatus* s.l. collected along the west coast of Africa, near Cadiz Spain, and in the Bay of Biscay suggests a single, genetically-cohesive species population along the margins of the NE and SE Atlantic Ocean. A seasonal ontogenetic migration strategy associated with complex oceanographic processes may contribute to the species' dispersion throughout this extended region (John et al., 1998; Peterson, 1998).

In the NE Atlantic, *C. carinatus* s.l. has been recorded along the west coast of the Iberian Peninsula, in the Bay of Biscay, and in waters southwest of the British Isles (Ceballos et al., 2004; John et al., 1998). Diapausing juveniles (copepodite stage CV) are transported from off the west coast of Africa to the Bay of Biscay by along-slope undercurrents (John et al., 1998), in which the species has established a permanent population (Ceballos and Álvarez-Marqués, 2006). Although the species has been traditionally considered an African upwelling specialist (Peterson, 1998), data concerning its reproductive rates in the NW Iberian coast and the Bay of Biscay show that *C. carinatus* s.l. can adopt different life strategies to cope with unusual or rare environmental conditions (Ceballos and Álvarez-Marqués, 2006; Ceballos et al., 2004).

Along the west coast of Africa, *C. carinatus* s.l. typically occurs in upwelling conditions (Thiriot, 1978), which show considerable latitudinal variability in this region in frequency, intensity and seasonality (Verheye et al., 2005). In coastal waters of the Guinea Current, *C. carinatus* s.l. is found during the short (3–4 months) upwelling season, whereas in the Benguela System (where the upwelling season extends over 6–8 months), the species maintains relatively high densities on the shelf throughout the year. In this region, populations are transported offshore in the wind-driven upwelling plume (Verheye et al., 2005). When productivity of the plume decreases, the juveniles (stage CV) descend to depths below 400 m and survive on lipid stores accumulated during the upwelling event (Arashkevich and Drits,

1996; Kosobokova et al., 1988), thereby reducing their metabolic activity and energy demands by as much as 96% (Auel et al., 2005). At the onset of a new upwelling period, the copepods are transported onto the shelf, where they molt to the adult stage and reproduce, remaining in surface waters and taking full advantage of the developing phytoplankton bloom (Verheye et al., 1992, 2005) to feed and accumulate lipid reserves.

The permanent reservoir of sub-adults of *C. carinatus* s.l. in deep waters along the Benguela System allows reseeding of extensive areas by advection over large spatial scales (Arashkevich and Drits, 1996; Peterson, 1998; Verheye et al., 1992; Verheye et al., 2005). The continuous and permanent poleward-flowing undercurrents along the continental slope off Africa may transport *C. carinatus* s.l. from the subtropical and tropical eastern Atlantic toward the British Isles (John et al., 2000), the northern limit of the species' distribution area. Similarly, the deep (600 m) northward-flowing Mediterranean Outflow Water (off the western Iberian Peninsula) may be a transport vehicle for the species (John et al., 1998). These oceanographic mechanisms – coupled with the vertical migration pattern of the species – may facilitate mixing of NE and SE Atlantic populations.

4.4. *C. carinatus* s.s. in the SW Atlantic

The molecular phylogeographic results presented here suggest that the distribution of *C. carinatus* s.s. is restricted to the SW Atlantic Ocean from 20°S, off Brazil to 47°S off Argentina. Similar results were found by Sabatini et al. (2007) on the basis of a morphological comparison of specimens of the species from off Brazil and Argentina. Near the northern limit of the species distribution, the area around Cape Frío, Brazil is characterized by a coastal upwelling, which occurs primarily during austral summer, when northeast winds drive nutrient-rich bottom South Atlantic Central Water (SACW) up onto the shelf. A bend in the coastline generates meanders in the Brazil Current that also drive upwelling and seasonal intrusions of SACW (Campos et al., 1999). *Calanoides carinatus* s.s. is found throughout the year in the Cape Frío region (Monteiro-Ribas et al., 1979), but its life cycle seems to be strongly linked with upwelling events (Lopes et al., 1999). The highest densities (600 to 1000 m⁻³) have been recorded in summer during periods of strongest upwelling intensity of SACW. Interestingly, the higher abundance levels of *C. carinatus* s.s. off Cape Frío are similar to those observed for *C. carinatus* s.l. in the Benguela upwelling region (Lopes et al., 1999). Farther south (23°S to 34°S), the species is found throughout the year, but is typically less abundant (0.1–530 m⁻³) than in the Cabo Frío region (Campaner, 1985; Campaner and Honda, 1987; Muelbert et al., 2008; Valentin and Monteiro Ribas, 1993). In the Argentine Sea, *C. carinatus* s.s. is widely distributed in coastal and inner shelf waters between 42° and 45°S. Abundance levels are similar to those of the southern Brazilian shelf (up to 500 m⁻³), with higher values typically recorded in summer (Ramírez and Sabatini, 2000). The highest abundances of the species are associated with frontal systems, including the estuarine front of the La Plata River (Viñas et al., 2002); the mid-shelf thermal front between coastal and shelf waters off Buenos Aires (Marrari et al., 2004; Santos and Ramírez, 1991); and tidal fronts along northern Patagonia (Sabatini and Martos, 2002). Throughout this distributional range, the species has not been reported in oceanic waters, since sampling has been done only in near-surface (<200 m)

depths of the water column (Campaner, 1985; Ramírez and Sabatini, 2000; Valentin and Monteiro Ribas, 1993). Thus, we do not yet know about the occurrence of diapausing copepods in the deeper strata (below 400 m depth) typical of this and other upwelling species (Peterson, 1998; Verheye et al., 2005).

We may hypothesize that no oceanographic barriers separate or isolate the populations of *C. carinatus* s.s. in the SW Atlantic Ocean. We may further speculate that abundant populations originating off Cape Frío, Brazil, during the summer upwelling period may be transported southwards to shelf areas off northern Argentina by the Subtropical Shelf Waters (Piola et al., 2000). On the basis of oceanographic, meteorological and biological observations, it has been suggested that the mean flow over the Argentinian Shelf reverses seasonally; flow toward the south-southwest during spring-summer (Martos and Piccolo, 1988; Negri et al., 1992; Piola et al., 2000) may allow transport of the species southward; while during winter, north-northeast flow of the Sub-Antarctic Waters, with added riverine outflows, (Möller and Piola, 2008; Piola et al., 2000), may carry populations toward the northern limit of their distribution on the Brazilian Shelf.

4.5. Conclusions and future studies

Phylogeographic analysis of samples collected throughout the NE/SE and SW Atlantic Ocean revealed that *C. carinatus* s.l. comprises two distinct species, with distributions along the ocean margins. Since the type locality of the species is in Brazilian waters, the SW Atlantic type is considered to represent *C. carinatus* s.s., with a biogeographical range restricted to SW Atlantic Ocean (20°S to 47°S). The NE/SE Atlantic type is most likely an undescribed, cryptic sibling species. The correct morphological identification and description of the latter species remains a future challenge for zooplankton researchers.

Further studies are needed to fully document the population dynamics and life history of *C. carinatus* s.s. in light of our conclusions that the species exhibits a much more restricted geographical range. It is critical to understand the species' ecological role in the coastal, continental shelf, and open ocean marine ecosystems of the SW Atlantic.

This work was possible because of the coordinated efforts of expert morphological taxonomists and molecular biologists and it is desirable that similar future projects can be developed in other sea areas where *C. carinatus* s.l. has been reported to occur.

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