Supplementary Material

Contrasting global genetic patterns in two biologically similar, widespread and invasive *Ciona* species (Tunicata, Ascidiacea)

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Supplementary Note. Taxonomic history of *C. robusta* and *C. intestinalis* and details of the historical observation of the two species, following literature report, in their introduced ranges at worldwide scale.

Until September 2015, the nominal species *Ciona intestinalis* was considered a species complex that included four cryptic species named *C. intestinalis* type A to type D[1,2]. *C. intestinalis* type A and type B each have disjunct distributions and are considered as invasive species in several regions of the world[2]. During the period of absence of taxonomic assignation, the native ranges of these two types were debated[2-4]. However, recent alpha-taxonomic works[5,6] showed that *C. intestinalis* type A matched with the description of *C. robusta* Hoshino & Tokioka, 1967[7] (ecotype from Onagawa, Japan) and *C. intestinalis* type B with the description of *C. intestinalis* (Linnaeus, 1767) *sensu* Millar[8] (ecotype from Millport, Scotland). *C. robusta* had been placed synonymy with *C. intestinalis* by Hoshino & Nishikawa in 1985[9]. The classification of *C. intestinalis* type A and *C. intestinalis* type B as *C. robusta* and *C. intestinalis* has been accepted in WoRMS since September 2015. They are accepted as native to the region where they were described; the NW Pacific for *C. robusta* and the NE Atlantic for *C. intestinalis.*

C. robusta exhibits a disjunct global distribution in warm-temperate regions; this distribution and the fact that the species is most often restricted to urban habitats (ports, marinas) has been explained by introduction events in many regions, as listed below:

- English Channel: *C. robusta* (first reported as *C. intestinalis* type A) was first reported in the early 2000s in this range[10]. This species is well established along the eastern Brittany coastline[11], like several other ascidians recently introduced in this range, e.g. *Asterocarpa humilis*, *Corella eumyota*[12].
- Mediterranean Sea: Before the taxonomic re-evaluation in September 2015, the specimens were assigned to *C. intestinalis* and considered as members of a cryptogenic species[13,14]. The first report dates back to the end of the 19th century by Roule in the harbor of Marseille[15]. So far, *C. robusta* and other species of the genus *Ciona* (e.g. *C. edwardsi* and *C. roulei*) have been reported in the Mediterranean Sea but not *C. intestinalis sensu stricto*.
- SE Pacific: The first report of *C. robusta* (with the name *C. intestinalis* used until the recognition of *C. robusta* as a valid species) in this region is debated. Individuals classified as *C. intestinalis* were recorded in the Magellan Strait (Magellanic Province) in 1885 by Traustedt[16]. However, it is likely that this report correspond to *C. antarctica* Hartmeyer, 1911 and not *C. robusta*[17,18], considering that the Magellanic Province has a temperate-subantarctic biota whereas *C. robusta* lives in warm-temperate regions[19]. The next known report of *Ciona* was made by Van Name in 1949[20] in Antofagasta Bay (Peruvian Province; warm-temperate). More recent surveys[17] of ascidians carried out in the 2010s in the Magellan Strait and around Coquimbo (Peruvian Province) confirmed the absence of both *C. robusta* and *C. intestinalis* in the Magellanic Province and the presence of only *C. robusta* in the Peruvian Province. We can thus reasonably consider that the observation by Van

Name was the first report of *C. robusta* and that the presence of this species in Chile dates back (at least) to the mid-20th century.

- NE Pacific: Specimens reported under the name of *C. intestinalis* were first recorded in San Diego Bay in early part of the 20th century by Ritter & Forsyth[21] (cited by Lambert & Lambert[22]). Since the two species (or types) have been distinguished, only *C. robusta* (*C. intestinalis* type A) has been reported in this region. The species is considered to be non-native in this region [4,22-24].
- South Africa: *C. robusta* (first reported under the name of *C. intestinalis*) was reported for the first time in South Africa in the mid-20th century [25,26]. The species is identified as non-native in this region[27-29].
- Oceania: As in South Africa, *C. robusta* was reported under the name *C. intestinalis* in the mid-20th century in the Port Phillip (Victoria) in Australia by Millar[30]. The species is considered an invasive species in harbours of the southern coastline of Australia[31,32]. *C. robusta* was also reported in New Zealand (as *C. intestinalis*) during the second part of the 20th century[33].

Currently, *C. intestinalis* displays a disjunct distribution in the N Atlantic (i.e. reported in both E and W coasts but absent from Artic coastal regions) and it has been reported in one region outside the N Atlantic (see below).

- NW Atlantic: *C. intestinalis* was first reported in the Gulf of St Lawrence by Van Name[34]. The species is currently distributed from Rhode Island to Newfoundland[35] and is found at high density on artificial substrates along the south coast of Nova Scotia[36] and eastern coasts of Prince Edwards Island[37]. The recent proliferation of *C. intestinalis* in this region earned it the status of invasive species in most studies (e.g.[38-42]). The non-native status of *C. intestinalis* is however debated in this region (i.e. cryptogenic status) as for several other marine invertebrates presenting a similar distribution[43].
- NW Pacific: *C. intestinalis* (reported as *C. intestinalis* type B) was recorded very recently on the western coastline of Bohai Bay and Yellow Seas by Zhan et al.[2]. The lack of genetic differentiation between North American, European and Asian populations supported its classification as a non-native species in the NW Pacific. It is important to note that *C. robusta* (reported as *C. intestinalis* type A) has been reported in the East Sea and Korea Strait but not yet in the Yellow Sea[44] _ENREF_43.

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Table S1. Sampling locations and details of genetic diversity indices of *Ciona robusta* and *C. intestinalis* computed from the mitochondrial COX3-ND1 sequences dataset (source: this study and Zhan et al.[25]).

The English Channel is the only sympatric region wherein the two species were reported. S: syntopic localities, i.e. wherein the two species coexist in the same habitat; - : localities where *C. robusta* has never been reported so far (most recent surveys in autumn 2014, JDDB, pers. obs.).

Nind: the number of individuals studied; nf: not found. Indices: Nh: number of haplotypes; Rh: haplotypic richness, with rarefaction size within brackets; Npr: number of private haplotypes; Rpr: corrected number (for sampling size) of private haplotype; S: number of polymorphic sites; Hd: Haplotype diversity; π: nucleotide diversity.

			C. robusta								***C. intestinalis***								**Source**
Sampling location	**Code**	**Status**	**Nind**	**Nh**	**Rh[9]**	**Npr**	**Rpr[9]**	**S**	**Hd**	**π (10^2)**	**Nind**	**Nh**	**Nh[19]**	**Npr**	**Npr[19]**	**S**	**Hd**	**π (10^2)**	
North Eastern Atlantic	NEA																		
English Channel	EC																		
Brighton, UK	Bri	-	nf								23	9	8.8	2	2.0	17	0.636	0.519	
Shoreham, UK	Sho	-	nf								24	13	12.7	3	3.0	21	0.884	0.892	
Southsea, UK	Shs	-	nf								24	16	15.5	6	5.9	24	0.917	0.719	
Gosport, UK	Gpt	-	nf								23	14	13.7	6	5.9	23	0.881	0.932	
Southampton, UK	Sth	-	nf								21	10	10.0	2	2.1	17	0.776	0.672	
Lymington, UK	Lym	-	nf								22	13	12.8	3	3.0	18	0.840	0.466	
Poole Quay, UK	Poo	-	nf								24	12	11.6	4	3.9	20	0.844	0.789	
Torquay, UK	Tor	S	1	1	-	0	-	-	-	-	24	7	6.9	1	1.0	15	0.779	0.839	
Brixham, UK	Brx	-	nf								24	11	10.7	3	2.9	17	0.877	0.740	
Plymouth, UK	Ply	S	24	4	3.7	2	1.2	3	0.308	0.056	24	12	11.8	3	2.9	20	0.924	0.924	
Falmouth, UK	Fal	S	24	3	3.6	1	1.1	2	0.409	0.074	23	6	5.9	2	2.0	12	0.656	0.265	
Saint Vaast, Fr	StV	S	24	1	1.0	0	0.0	0	0.000	0.000	25	14	13.5	4	3.8	25	0.930	0.913	
Saint Malo, Fr	StM	S	23	1	1.0	0	0.0	0	0.000	0.000	19	9	9.0	1	1.0	14	0.801	0.745	This study
Saint Quay, Fr	StQ	S	24	3	2.9	2	1.9	2	0.424	0.079	24	8	7.8	4	3.6	17	0.808	0.689	
Perros Guirec, Fr	Per	S	24	3	2.9	1	0.7	2	0.163	0.029	26	11	10.6	2	2.0	20	0.871	0.959	
Trébeurden, Fr	Tre	S	24	1	1.0	0	0.0	0	0.000	0.000	24	10	9.8	2	2.0	18	0.870	0.974	
Roscoff-Bloscon, Fr	Blo	S	18	2	2.3	0	0.2	1	0.111	0.019	26	16	15.0	7	6.5	28	0.858	0.900	
Aber Wrac'h, Fr	AbW	-	nf								25	13	12.6	2	2.0	22	0.920	0.946	
Brest-Château, Fr	Cha	S	23	2	1.7	0	0.0	1	0.166	0.029	27	10	9.4	2	1.9	16	0.815	0.930	
Brest-Moulin Blanc, Fr	MBl	S	26	2	1.5	1	0.6	1	0.077	0.013	24	13	12.8	5	4.1	23	0.932	0.885	
Camaret, Fr	Cam	S	24	1	1.0	0	0.0	0	0.000	0.000	21	15	14.9	3	3.1	27	0.952	0.956	
Concarneau, Fr	Con	S	24	1	1.0	0	0.0	0	0.000	0.000	24	9	8.9	2	1.9	16	0.866	0.913	
Lorient, Fr	Lor	-	nf								24	10	9.7	2	2.0	19	0.793	0.877	
Crouesty, Fr	Cro	S	24	1	1.0	0	0.0	0	0.000	0.000	24	11	10.7	2	1.9	19	0.830	0.923	
Quiberon, Fr	Qui	S	13	2	1.9	0	0.4	1	0.154	0.027	23	9	8.8	2	2.0	15	0.723	0.460	
Total EC			*320*	*13*		*10*		*10*	*0.138*	*0.025*	*592*	*117*		*110*		*100*	*0.870*	*0.854*	
North Sea (Skagerrak)	NS																		
Grundsund, Sw	Grun										21	5	5.0	2	2.0	5	0.633	0.214	This study
Gullmar Fjord, Sw	GullF										22	5	5.0	2	2.0	8	0.532	0.231	

Fiskebäckskil, Sw	Fiske									24	8	7.8	2	2.0	7	0.659	0.224	
Total NS										*67*	*13*		*9*		*13*	*0.619*	*0.232*	
Total NEA		*320*	*13*		*10*		*10*	*0.138*	*0.025*	*659*	*126*		*122*		*103*	*0.854*	*0.815*	
North Western Atlantic	NWA																	
Cardigan River, Ca	CR									30	8	5.6	3	2.6	7	0.556	0.188	
Brudenell River, Ca	BR									30	3	2.9	1	0.9	2	0.191	0.037	
Murray River, Ca	MR									30	3	2.9	1	1.0	2	0.191	0.037	
Sydney, Ca	SD									42	5	3.6	0	0.2	9	0.577	0.195	
Point Tupper, Ca	PO									21	3	3.0	0	0.0	2	0.267	0.052	
Halifax, Ca	HF									28	4	3.9	0	0.0	7	0.429	0.308	
Chester, Ca	CT									28	4	3.8	2	1.8	5	0.418	0.124	[25]
Martin's River, Ca	MA									45	5	4.6	1	0.7	8	0.574	0.146	
Mahone Bay, Ca	MB									28	3	3.0	0	0.0	5	0.569	0.121	
Stone Hurst, Ca	ST									26	6	4.9	1	1.0	7	0.652	0.171	
Lunenburg, Ca	LU									21	3	3.0	0	0.1	3	0.338	0.079	
Shelburne, Ca	SB									39	7	5.6	1	1.0	13	0.547	0.386	
Port La Tour, Ca	PT									21	3	2.0	0	0.0	2	0.267	0.052	
Yarmouth, Ca	YM									20	8	6.0	2	2.0	14	0.821	0.619	
Nahant, US	Nah									24	6	5.9	2	1.9	16	0.630	0.740	This study
Groton, US	GT									48	6	5.6	0	0.0	12	0.688	0.607	[25]
Total NWA										*481*	*25*		*20*		*31*	*0.498*	*0.324*	
Mediterranean Sea	MedS																	
Naples, It	Napl	23	1	1.0	0	0.0	0	0.000	0.000									This study
Sete, Fr	Sete	21	3	2.4	0	0.2	2	0.186	0.033									
Total MedS		*44*	*3*		*0*		*2*	*0.090*	*0.016*									
North Western Pacific	NWP																	
Nishinomiya, Japan	Nishi	32	7	6.4	0	0.6	8	0.821	0.341									This study
Tokyo, Japan	Tokyo	32	6	5.7	1	0.9	7	0.758	0.261									
Total NWP		*64*	*9*		*1*		*9*	*0.792*	*0.301*									
North Eastern Pacific	NEP																	
north NEP	nNEP																	
Tomales Bay, US	TB	13	4	3.9	0	0.0	7	0.679	0.471									[25]
San Francisco Estuary, US	SF	9	5	5.0	1	1.0	9	0.861	0.597									[25]+This study
Monterey Bay, US	MO	18	6	5.4	0	1.1	7	0.791	0.275									[25]
Total nNEP		*40*	*9*		*2*		*10*	*0.837*	*0.457*									
south NEP	sNEP																	
Santa Barbara, US	SB	16	6	5.6	2	1.9	6	0.783	0.298									
Channel Islands, US	CI	27	8	6.0	2	1.4	12	0.772	0.434									
Port Hueneme, US	PH	21	8	6.3	3	2.1	13	0.752	0.465									
Los Angeles, US	LA	31	5	4.3	0	0.0	5	0.735	0.321									[25]
Newport Bay, US	NB	21	5	4.6	0	0.0	5	0.776	0.333									
Oceanside Estuary, US	OE	23	8	4.6	0	0.2	9	0.838	0.429									
Mission Bay, US	MI	8	3	-	0		4	0.714	0.322									
San Diego, US	SD	26	7	5.6	0	0.1	8	0.769	0.397									

Total sNEP		*173*	*16*		*12*		*20*	*0.775*	*0.393*									
Total NEP		*213*	*22*		*16*		*21*	*0.814*	*0.419*									
South Eastern Pacific	SEP																	
north SEP	nSEP																	
Antofagasta, Chile	Anto	3	3	-	0	-	3	1.000	0.346									
Coquimbo, Chile	Coqui	24	8	6.9	2	1.3	9	0.764	0.288									This study
Guanaqueros, Chile	Guana	24	5	3.8	1	0.6	8	0.685	0.244									
Total nSEP		*51*	*12*		*3*		*10*	*0.724*	*0.385*									
south SEP	sSEP																	
Talcahuano, Chile	Talca	9	3	3.0	1	1.0	4	0.556	0.25									This study
Puerto Montt, Chile	Mont	13	5	5.7	1	1.3	4	0.808	0.231									
Total sSEP		*22*	*8*		*2*		*5*	*0.771*	*0.246*									
Total SEP		*73*	*14*		*6*		*12*	*0.749*	*0.259*									
All dataset																		
Mean		**21.0**	**3.9**				**4.2**	**0.469**	**0.196**	**25.9**	**8.5**				**14.0**	**0.694**	**0.554**	
(SD)		**6.9**	**2.3**				**3.8**	**0.344**	**0.181**	**6.5**	**3.8**				**7.4**	**0.217**	**0.347**	
Total		**714**	**45**				**39**	**0.703**	**0.334**	**1140**	**147**				**114**	**0.737**	**0.635**	

Table S2. Number of *Ciona robusta* (A) and *C. intestinalis* (B) individuals identified in clusters of the haplotype network based on COX3-ND1 dataset.

Clusters were identified from the haplotype network built with COX3-ND1 mtDNA sequences and shown in Figure 3 in the main text.

(A) *Ciona robusta*

Sampling location	Code	C1	C2	Not in cluster	Total
North Eastern Atlantic	NEA				
English Channel	EC				
Torquay, UK	Tor	1	0	0	1
Plymouth, UK	Ply	24	0	0	24
Falmouth, UK	Fal	24	0	0	24
Saint Vaast, Fr	StV	24	0	0	24
Saint Malo, Fr	StM	23	0	0	23
Saint Quay, Fr	StQ	24	0	0	24
Perros Guirec, Fr	Per	24	0	0	24
Trébeurden, Fr	Tre	24	0	0	24
Roscoff-Bloscon, Fr	Blo	18	0	0	18
Brest-Château, Fr	Cha	23	0	0	23
Brest-Moulin Blanc, Fr	MBl	26	0	0	26
Camaret, Fr	Cam	24	0	0	24
Concarneau, Fr	Con	24	0	0	24
Crouesty, Fr	Cro	24	0	0	24
Quiberon, Fr	Qui	13	0	0	13
Total EC		*320*	*0*	*0*	*320*
Mediterranean Sea	MedS				
Naples, It	Napl	23	0	0	23
Sete, Fr	Sete	21	0	0	21
Total MedS		*44*	*0*	*0*	*44*
North Western Pacific	NWP				
Nishinomiya, Japan	Nishi	29	2	1	32
Tokyo, Japan	Tokyo	27	0	5	32
Total NWP		*56*	*2*	*6*	*64*
North Eastern Pacific	NEP				
north NEP	nNEP				
Tomales Bay, US	TB	9	4	0	13
San Francisco Estuary, US	SF	4	5	0	9
Monterey Bay, US	MO	13	5	0	18
Total nNEP		*26*	*14*	*0*	*40*
south NEP	sNEP				
Santa Barbara, US	SB	0	16	0	16
Channel Islands, US	CI	13	14	0	27
Port Hueneme, US	PH	9	12	0	21
Los Angeles, US	LA	6	25	0	31
Newport Bay, US	NB	7	14	0	21
Oceanside Estuary, US	OE	5	18	0	23
Mission Bay, US	MI	1	7	0	8
San Diego, US	SD	11	15	0	26
Total sNEP		*52*	*121*	*0*	*173*
Total NEP		*78*	*135*	*0*	*213*
South Eastern Pacific	SEP				
north SEP	nSEP				
Antofagasta, Chile	Anto	3	0	0	3
Coquimbo, Chile	Coqui	19	0	5	24
Guanaqueros, Chile	Guana	20	0	4	24
Total nSEP		*42*	*0*	*9*	*51*
south SEP	sSEP				

Talcahuano, Chile	Talca	9	0		0	9
Puerto Montt, Chile	Mont	13	0		0	13
Total sSEP		*22*	*0*		*0*	*22*
Total SEP		*64*	*0*		*9*	*73*
Total		**562**	**137**		**15**	**714**

(B) *C. intestinalis*

Sampling location	**Code**	**C1**	**C2**	**C3**	**Not in cluster**	**Total**
North Eastern Atlantic	NEA					
English Channel	EC					
Brighton, UK	Bri	19	3	1	0	23
Shoreham, UK	Sho	16	8	0	0	24
Southsea, UK	Shs	20	4	0	0	24
Gosport, UK	Gpt	16	6	1	0	23
Southampton, UK	Sth	19	2	0	0	21
Lymington, UK	Lym	19	3	0	0	22
Poole Quay, UK	Poo	17	7	0	1	24
Torquay, UK	Tor	16	5	3	0	24
Brixham, UK	Brx	19	3	2	0	24
Plymouth, UK	Ply	13	11	0	0	24
Falmouth, UK	Fal	22	1	0	0	23
Saint Vaast, Fr	StV	15	10	0	0	25
Saint Malo, Fr	StM	12	6	1	0	19
Saint Quay, Fr	StQ	19	1	4	0	24
Perros Guirec, Fr	Per	13	13	0	1	26
Trébeurden, Fr	Tre	13	8	3	0	24
Roscoff-Bloscon, Fr	Blo	18	6	2	0	26
Aber Wrac'h, Fr	AbW	15	8	2	0	25
Brest-Château, Fr	Cha	13	11	3	0	27
Brest-Moulin Blanc, Fr	MBl	15	8	1	0	24
Camaret, Fr	Cam	10	8	3	0	21
Concarneau, Fr	Con	16	7	1	0	24
Lorient, Fr	Lor	11	12	1	0	24
Crouesty, Fr	Cro	13	9	2	0	24
Quiberon, Fr	Qui	19	4	0	0	23
Total EC		*398*	*164*	*30*	*2*	*592*
North Sea (Skagerrak)	*NS*					
Grundsund, Sw	Grun	21	0	0	0	21
Gullmar Fjord, Sw	GullF	22	0	0	0	22
Fiskebäckskil, Sw	Fiske	24	0	0	0	24
Total NS		*67*	*0*	*0*	*0*	*67*
Total NEA		465	164	30	2	661
North Western Atlantic	NWA					
Cardigan River, Ca	CR	30	0	0	0	30
Brudenell River, Ca	BR	30	0	0	0	30
Murray River, Ca	MR	30	0	0	0	30
Sydney, Ca	SD	41	1	0	0	42
Point Tupper, Ca	PO	21	0	0	0	21
Halifax, Ca	HF	25	3	0	0	28
Chester, Ca	CT	28	0	0	0	28
Martin's River, Ca	MA	45	0	0	0	45
Mahone Bay, Ca	MB	28	0	0	0	28
Stone Hurst, Ca	ST	26	0	0	0	26
Lunenburg, Ca	LU	21	0	0	0	21
Shelburne, Ca	SB	35	4	0	0	39
Port La Tour, Ca	PT	21	0	0	0	21
Yarmouth, Ca	YM	17	3	0	0	20
Nahant, US	Nah	18	6	0	0	24
Groton, US	GT	37	11	0	0	48
Total NWA		453	28	0	0	481

Total	**918**	**192**	**30**			**2**	**1140**

Table S3. Sampling locations and details of genetic diversity indices of *Ciona robusta* and *C. intestinalis* computed from the concatenated sequences dataset.

Indices were computed over concatenated mitochondrial DNA sequences (COI and COX3-ND1).

S: syntopic locality (see legend of Supplementary Table S1).

Nind: the number of individuals studied; nf: not found. Indices: Nh: number of haplotypes; Npr: number of private haplotypes; Hd: Haplotype diversity; π: nucleotide diversity.

			C. robusta					*C. intestinalis*				
Sampling location	**Code**	**Status**	**Nind**	**Nh**	**Npr**	**Hd**	**$\pi(10^2)$**	**Nind**	**Nh**	**Npr**	**Hd**	**$\pi(10^2)$**
North Eastern Atlantic	NEA											
English Channel	EC											
Brighton, UK	Bri	-	nf					23	15	4	0.925	0.453
Shoreham, UK	Sho	-	nf					24	18	5	0.967	0.753
Southsea, UK	Shs	-	nf					24	19	10	0.978	0.528
Gosport, UK	Gpt	-	nf					23	20	9	0.976	0.718
Southampton, UK	Sth	-	nf					21	17	6	0.976	0.549
Lymington, UK	Lym	-	nf					22	18	6	0.978	0.428
Poole Quay, UK	Poo	-	nf					24	20	7	0.975	0.661
Torquay, UK	Tor	S	1	1	0	-	-	24	11	4	0.870	0.715
Brixham, UK	Brx	-	nf					24	16	4	0.946	0.607
Plymouth, UK	Ply	S	24	5	2	0.377	0.056	24	18	9	0.964	0.708
Falmouth, UK	Fal	S	24	5	1	0.659	0.112	23	15	7	0.909	0.311
Saint Vaast, Fr	StV	S	24	2	0	0.159	0.011	25	18	6	0.963	0.692
Saint Malo, Fr	StM	S	23	1	0	0.000	0.000	19	13	3	0.924	0.657
Saint Quay, Fr	StQ	S	24	7	4	0.667	0.078	24	13	7	0.931	0.592
Perros Guirec, Fr	Per	S	24	3	1	0.163	0.012	26	21	6	0.985	0.776
Trébeurden, Fr	Tre	S	24	2	1	0.083	0.006	24	17	4	0.967	0.788
Roscoff-Bloscon, Fr	Blo	S	18	2	1	0.111	0.016	26	23	10	0.982	0.731
Aber Wrac'h, Fr	AbW	-	nf					25	19	6	0.977	0.769
Brest-Château, Fr	Cha	S	23	2	0	0.166	0.012	27	21	8	0.977	0.784
Brest-Moulin Blanc, Fr	MBl	S	26	3	1	0.151	0.011	24	19	9	0.975	0.729
Camaret, Fr	Cam	S	24	1	0	0.000	0.000	21	20	7	0.995	0.722
Concarneau, Fr	Con	S	24	2	0	0.159	0.011	24	16	4	0.957	0.697
Lorient, Fr	Lor	-	nf					24	19	7	0.967	0.696
Crouesty, Fr	Cro	S	24	2	0	0.489	0.035	24	19	4	0.978	0.769
Quiberon, Fr	Qui	S	13	4	0	0.526	0.062	23	18	5	0.957	0.403
Total EC			*320*	*18*	*13*	*0.285*	*0.033*	*592*	*229*	*157*	*0.979*	*0.692*
North Sea (Skagerrak)	NS											
Grundsund, Sw	Grun							21	10	6	0.871	0.244
Gullmar Fjord, Sw	GullF							22	10	5	0.857	0.228
Fiskebäckskil, Sw	Fiske							24	10	4	0.851	0.184
Total NS								*67*	*22*	*15*	*0.901*	*0.237*
Total NEA			*320*	*18*	*13*	*0.285*	*0.033*	*659*	*248*	*172*	*0.977*	*0.666*
North Western Atlantic	NWA											
Nahant, US	Nah							24	12	7	0.917	0.632
Mediterranean Sea	MedS											
Naples, It	Napl		23	2	0	0.166	0.012					
Sete, Fr	Sete		21	11	8	0.910	0.143					
Total MedS			*44*	*11*	*8*	*0.642*	*0.079*					
North Western Pacific	NWP											
Nishinomiya, Japan	Nishi		32	9	2	0.845	0.454					
Tokyo, Japan	Tokyo		32	8	1	0.774	0.34					
Total NWP			*64*	*11*	*3*	*0.813*	*0.398*					
South Eastern Pacific	SEP											
North SEP	*nSEP*											
Antofagasta, Chile	Anto		3	3	1	1.000	0.333					
Coquimbo, Chile	Coqui		24	13	4	0.902	0.387					
Guanaqueros, Chile	Guana		24	12	2	0.913	0.354					
Total nSEP			51	17	7	0.905	0.360					
South SEP	*sSEP*											
Talcahuano, Chile	Talca		9	6	2	0.833	0.345					
Puerto Montt, Chile	Mont		13	7	1	0.846	0.397					

Total sSEP	*sSEP*	*22*	*10*	*3*	*0.879*	*0.404*					
Total SEP		*73*	*21*	*11*	*0.898*	*0.377*					
All dataset											
Mean		**21**	**5**		**0.454**	**0.132**	**24**	**17**		**0.948**	**0.611**
(SD)		**8**	**4**		**0**	**0**	**2**	**4**		**0**	**0**
Total		**501**	**48**		**0.598**	**0.225**	**683**	**255**		**0.977**	**0.665**

Table S4. Number of *Ciona intestinalis* individuals identified in clusters C1, C2 and C3 of the haplotype network based on concatenated dataset.

Clusters were identified from the haplotype network built with concatenated mtDNA sequences (COI and COX3-ND1) and shown in Supplementary Figure S1.

Sampling location	**Code**	**C1**	**C2**	**C3**	**Not in cluster**	**Total**
North Eastern Atlantic	NEA					
English Channel	EC					
Brighton, UK	Bri	19	3	1	0	23
Shoreham, UK	Sho	17	7	0	0	24
Southsea, UK	Shs	20	4	0	0	24
Gosport, UK	Gpt	15	6	1	1	23
Southampton, UK	Sth	17	4	0	0	21
Lymington, UK	Lym	20	2	0	0	22
Poole Quay, UK	Poo	18	6	0	0	24
Torquay, UK	Tor	16	5	3	0	24
Brixham, UK	Brx	20	2	2	0	24
Plymouth, UK	Ply	14	9	0	1	24
Falmouth, UK	Fal	22	1	0	0	23
Saint Vaast, Fr	StV	16	9	0	0	25
Saint Malo, Fr	StM	16	2	1	0	19
Saint Quay, Fr	StQ	18	1	5	0	24
Perros Guirec, Fr	Per	16	10	0	0	26
Trébeurden, Fr	Tre	13	8	3	0	24
Roscoff-Bloscon, Fr	Blo	18	4	2	2	26
Aber Wrac'h, Fr	AbW	18	5	2	0	25
Brest-Château, Fr	Cha	16	8	3	0	27
Brest-Moulin Blanc, Fr	MBl	19	4	1	0	24
Camaret, Fr	Cam	8	9	4	0	21
Concarneau, Fr	Con	16	6	1	1	24
Lorient, Fr	Lor	15	8	1	0	24
Crouesty, Fr	Cro	16	6	2	0	24
Quiberon, Fr	Qui	21	2	0	0	23
Total EC		*424*	*131*	*32*	*5*	*592*
North Sea (Skagerrak)	NS					
Grundsund, Sw	Grun	21	0	0	0	21
Gullmar Fjord, Sw	GullF	22	0	0	0	22
Fiskebäckskil, Sw	Fiske	24	0	0	0	24
Total NS		*67*	*0*	*0*	*0*	*67*
Total NEA		491	131	32	5	659
North Western Atlantic	NWA					
Nahant, US	Nah	19	5	0	0	24
Total		**510**	**136**	**32**	**5**	**683**

Table S5. Estimates of pairwise population genetic differentiation for *Ciona robusta* (A) and *C. intestinalis* (B) based on COX3-ND1 sequences. The fixation index ϕ_{ST} was computed based on COX3-ND1 mitochondrial DNA sequences with the software Arlequin v 3.5. Bold numbers indicate statistical significance (*P*-value <0.05). Population labels are detailed in Supplementary Table S1.

(A) *C. robusta*

	Ply	Fal	StV	StM	StQ	Per	Tre	Blo	Cha	MBl	Cam	Con	Cro	Qui	Napl	Set	Coqui	Guana	Talca	Mont	Nishi	Toky	TB	SF	MO	SB	CI	PH	LA	NB	OH
Ply																															
Fal	0.067																														
StV	0.050	**0.267**																													
StM	0.047	**0.261**	0.000																												
StQ	**0.067**	**0.215**	0.087	0.084																											
Per	0.039	**0.235**	0.000	-0.002	0.065																										
Tre	0.050	**0.267**	0.000	0.000	0.087	0.000																									
Blo	0.030	**0.219**	0.016	0.014	0.059	-0.003	0.016																								
Cha	0.047	**0.236**	0.048	0.045	**0.075**	0.023	0.048	0.025																							
MBl	0.042	**0.244**	-0.003	-0.005	**0.069**	0.000	-0.003	-0.003	-0.017																						
Cam	0.050	**0.267**	0.000	0.000	0.087	0.000	0.000	0.016	0.048	-0.003																					
Con	0.050	**0.267**	0.000	0.000	0.087	0.000	0.000	0.016	0.048	-0.003	0.000																				
Cro	0.050	**0.267**	0.000	0.000	0.087	0.000	0.000	0.016	0.048	-0.003	0.000	0.000																			
Qui	0.017	**0.189**	0.050	0.047	0.044	-0.043	0.050	0.004	0.023	0.000	0.050	0.050	0.050																		
Napl	0.047	**0.261**	0.000	0.000	0.084	-0.002	0.000	0.014	0.045	-0.005	0.000	0.000	0.000	0.047																	
Sete	0.034	**0.221**	0.007	0.004	0.059	0.000	0.007	-0.038	-0.025	-0.021	0.007	0.007	0.007	-0.003	0.004																
Coqui	**0.332**	**0.298**	**0.401**	**0.395**	**0.368**	**0.386**	**0.401**	**0.356**	**0.382**	**0.391**	**0.401**	**0.401**	**0.401**	**0.319**	**0.395**	**0.368**															
Guana	**0.424**	**0.365**	**0.507**	**0.501**	**0.465**	**0.489**	**0.507**	**0.460**	**0.485**	**0.498**	**0.507**	**0.507**	**0.507**	**0.422**	**0.501**	**0.471**	-0.018														
Talca	**0.202**	**0.147**	**0.389**	**0.380**	**0.279**	**0.334**	**0.389**	**0.303**	**0.331**	**0.350**	**0.389**	**0.389**	**0.389**	**0.245**	**0.380**	**0.308**	0.014	0.065													
Mont	**0.430**	**0.366**	**0.563**	**0.555**	**0.476**	**0.525**	**0.563**	**0.493**	**0.520**	**0.538**	**0.563**	**0.563**	**0.563**	**0.440**	**0.555**	**0.501**	-0.019	-0.020	0.018												
Nishi	**0.439**	**0.395**	**0.496**	**0.491**	**0.470**	**0.484**	**0.496**	**0.458**	**0.481**	**0.494**	**0.496**	**0.496**	**0.496**	**0.426**	**0.491**	**0.469**	**0.044**	0.016	**0.110**	0.013											
Tokyo	**0.482**	**0.436**	**0.544**	**0.539**	**0.513**	**0.531**	**0.544**	**0.506**	**0.527**	**0.539**	**0.544**	**0.544**	**0.544**	**0.475**	**0.539**	**0.516**	**0.048**	0.011	**0.154**	0.003	-0.001										
TB	**0.641**	**0.614**	**0.695**	**0.688**	**0.654**	**0.679**	**0.695**	**0.643**	**0.673**	**0.688**	**0.695**	**0.695**	**0.695**	**0.595**	**0.688**	**0.658**	**0.346**	**0.379**	**0.398**	**0.369**	**0.370**	**0.399**									
SF	**0.670**	**0.638**	**0.732**	**0.725**	**0.684**	**0.713**	**0.732**	**0.675**	**0.707**	**0.723**	**0.732**	**0.732**	**0.732**	**0.621**	**0.725**	**0.691**	**0.328**	**0.366**	**0.387**	**0.350**	**0.347**	**0.371**	-0.033								
MO	**0.585**	**0.518**	**0.668**	**0.662**	**0.619**	**0.649**	**0.668**	**0.619**	**0.644**	**0.659**	**0.668**	**0.668**	**0.668**	**0.578**	**0.662**	**0.630**	**0.110**	**0.055**	**0.231**	**0.101**	**0.053**	**0.054**	**0.320**	**0.293**							
SB	**0.788**	**0.764**	**0.835**	**0.831**	**0.797**	**0.820**	**0.835**	**0.801**	**0.817**	**0.826**	**0.835**	**0.835**	**0.835**	**0.772**	**0.831**	**0.808**	**0.542**	**0.577**	**0.624**	**0.592**	**0.565**	**0.579**	**0.311**	**0.146**	**0.520**						
CI	**0.468**	**0.445**	**0.507**	**0.501**	**0.486**	**0.498**	**0.507**	**0.467**	**0.493**	**0.504**	**0.507**	**0.507**	**0.507**	**0.431**	**0.501**	**0.481**	**0.186**	**0.199**	**0.244**	**0.188**	**0.220**	**0.209**	**0.132**	0.035	**0.160**	**0.186**					
PH	**0.498**	**0.467**	**0.544**	**0.538**	**0.517**	**0.533**	**0.544**	**0.497**	**0.528**	**0.541**	**0.544**	**0.544**	**0.544**	**0.457**	**0.538**	**0.513**	**0.204**	**0.213**	**0.257**	**0.203**	**0.241**	**0.227**	**0.164**	0.062	**0.150**	**0.156**	-0.025				
LA	**0.633**	**0.617**	**0.666**	**0.662**	**0.643**	**0.657**	**0.666**	**0.633**	**0.653**	**0.662**	**0.666**	**0.666**	**0.666**	**0.604**	**0.662**	**0.643**	**0.366**	**0.392**	**0.446**	**0.392**	**0.388**	**0.389**	**0.209**	0.035	**0.340**	**0.093**	0.037	0.048			
NB	**0.595**	**0.569**	**0.642**	**0.637**	**0.610**	**0.629**	**0.642**	**0.598**	**0.625**	**0.636**	**0.642**	**0.642**	**0.642**	**0.560**	**0.637**	**0.612**	**0.272**	**0.298**	**0.356**	**0.295**	**0.298**	**0.297**	**0.174**	0.024	**0.252**	**0.161**	-0.015	0.003	-0.021		
OH	**0.599**	**0.580**	**0.637**	**0.632**	**0.610**	**0.626**	**0.637**	**0.596**	**0.622**	**0.633**	**0.637**	**0.637**	**0.637**	**0.560**	**0.632**	**0.609**	**0.341**	**0.371**	**0.398**	**0.366**	**0.379**	**0.384**	**0.194**	0.044	**0.328**	0.053	0.023	0.024	-0.011	-0.003	
SD	**0.520**	**0.499**	**0.559**	**0.553**	**0.534**	**0.549**	**0.559**	**0.518**	**0.544**	**0.555**	**0.559**	**0.559**	**0.559**	**0.482**	**0.553**	**0.532**	**0.237**	**0.256**	**0.302**	**0.251**	**0.277**	**0.269**	**0.174**	0.051	**0.222**	**0.142**	-0.025	-0.020	0.015	-0.020	-0.013

(B) *C. intestinalis*

	Bri	Sho	Shs	Gpt	Sth	Lym	Poo	Tor	Brx	Ply	Fal	StV	StM	StQ	Per	Tre	Blo	AbW	Cha	MBl	Cam	Con	Lor	Cro	Qui	Grun	GullF	Fiske
Sho	0.040																											
Shs	-0.027	0.014																										
Gpt	0.025	-0.028	0.005																									
Sth	-0.020	0.004	-0.024	-0.013																								
Lym	-0.024	0.078	-0.013	0.061	-0.002																							
Poo	0.012	-0.023	-0.008	-0.023	-0.015	0.039																						
Tor	0.051	0.026	0.037	0.015	0.039	**0.088**	0.025																					
Brx	0.011	0.052	0.015	0.033	0.021	0.036	0.037	-0.001																				
Ply	**0.133**	-0.005	**0.090**	0.008	**0.080**	**0.178**	0.021	0.049	**0.118**																			
Fal	0.049	**0.162**	0.056	**0.140**	0.074	0.031	**0.126**	**0.163**	**0.091**	**0.270**																		
StV	0.057	-0.030	0.030	-0.022	0.019	**0.098**	-0.009	0.031	**0.058**	-0.010	**0.175**																	
StM	0.007	-0.031	-0.012	-0.038	-0.018	0.049	-0.034	-0.011	-0.002	0.015	**0.140**	-0.021																
StQ	0.047	**0.097**	0.046	**0.067**	0.056	**0.067**	**0.076**	-0.003	-0.013	**0.155**	**0.133**	**0.106**	0.037															
Per	**0.129**	-0.007	**0.088**	0.004	0.077	**0.174**	0.021	0.062	**0.117**	-0.024	**0.260**	-0.018	0.011	**0.164**														
Tre	**0.092**	-0.005	0.065	-0.014	0.049	**0.141**	0.013	0.023	**0.057**	0.002	**0.220**	-0.009	-0.019	**0.095**	-0.008													
Blo	0.002	-0.017	-0.008	-0.024	-0.014	0.036	-0.025	0.009	0.012	0.029	**0.094**	-0.007	-0.040	0.046	0.026	-0.001												
AbW	0.051	-0.027	0.028	-0.026	0.016	**0.092**	-0.016	0.019	0.049	-0.004	**0.160**	-0.023	-0.032	**0.086**	-0.010	-0.021	-0.019											
Cha	**0.128**	0.002	**0.092**	0.004	**0.082**	**0.180**	0.023	0.053	**0.105**	-0.014	**0.267**	0.001	0.005	**0.144**	-0.015	-0.023	0.019	-0.014										
MBl	0.003	-0.025	-0.012	-0.035	-0.024	0.036	-0.031	0.012	0.013	0.022	**0.117**	-0.018	-0.042	0.050	0.020	-0.003	-0.032	-0.023	0.017									
Cam	**0.176**	0.034	**0.136**	0.036	**0.128**	**0.227**	0.068	**0.075**	**0.137**	-0.002	**0.309**	0.016	0.042	**0.170**	-0.008	-0.010	0.053	0.013	-0.020	0.052								
Con	**0.150**	0.001	**0.106**	0.011	0.094	**0.201**	0.030	0.070	**0.139**	-0.032	**0.297**	-0.005	0.025	**0.177**	-0.026	-0.003	0.034	-0.003	-0.022	0.027	-0.011							
Lor	**0.189**	0.017	**0.140**	0.031	**0.125**	**0.241**	0.052	**0.091**	**0.161**	-0.025	**0.334**	0.008	0.043	**0.206**	-0.020	0.003	0.056	0.008	-0.021	0.050	-0.016	-0.029						
Cro	**0.096**	-0.019	0.065	-0.016	0.052	**0.148**	0.004	0.034	**0.078**	-0.021	**0.234**	-0.024	-0.016	**0.123**	-0.027	-0.031	-0.002	-0.026	-0.029	-0.006	-0.017	-0.025	-0.018					
Qui	-0.026	0.048	-0.020	0.034	-0.018	-0.020	0.020	0.068	0.020	**0.149**	0.045	**0.067**	0.014	**0.058**	**0.141**	**0.105**	0.013	0.059	**0.146**	0.011	**0.195**	**0.169**	**0.202**	**0.112**				
Grun	**0.089**	**0.210**	**0.096**	**0.186**	**0.114**	**0.071**	**0.175**	**0.204**	**0.128**	**0.315**	**0.125**	**0.218**	**0.197**	**0.165**	**0.301**	**0.264**	**0.148**	**0.217**	**0.313**	**0.164**	**0.348**	**0.344**	**0.381**	**0.281**	**0.073**			
GullF	**0.060**	**0.195**	**0.068**	**0.169**	**0.094**	0.026	**0.156**	**0.189**	**0.107**	**0.305**	**0.077**	**0.204**	**0.177**	**0.136**	**0.297**	**0.253**	**0.131**	**0.203**	**0.302**	**0.140**	**0.341**	**0.333**	**0.372**	**0.270**	0.051	**0.049**		
Fiske	**0.063**	**0.201**	**0.069**	**0.174**	**0.095**	0.026	**0.161**	**0.197**	**0.113**	**0.313**	**0.082**	**0.213**	**0.186**	**0.139**	**0.305**	**0.262**	**0.136**	**0.210**	**0.310**	**0.146**	**0.351**	**0.339**	**0.382**	**0.279**	0.050	**0.047**	-0.018	
CR	0.039	**0.195**	**0.062**	**0.166**	**0.086**	0.024	**0.149**	**0.186**	**0.100**	**0.315**	**0.081**	**0.208**	**0.172**	**0.138**	**0.301**	**0.254**	**0.121**	**0.200**	**0.305**	**0.140**	**0.355**	**0.344**	**0.384**	**0.272**	0.036	**0.094**	**0.054**	**0.060**
BR	**0.086**	**0.254**	**0.100**	**0.224**	**0.138**	**0.048**	**0.209**	**0.246**	**0.145**	**0.377**	**0.114**	**0.262**	**0.259**	**0.205**	**0.364**	**0.318**	**0.172**	**0.260**	**0.369**	**0.202**	**0.418**	**0.411**	**0.453**	**0.339**	**0.080**	**0.159**	**0.052**	**0.058**
MR	**0.079**	**0.248**	**0.095**	**0.218**	**0.132**	**0.043**	**0.202**	**0.240**	**0.139**	**0.372**	**0.110**	**0.257**	**0.251**	**0.197**	**0.359**	**0.312**	**0.166**	**0.254**	**0.363**	**0.195**	**0.413**	**0.406**	**0.447**	**0.333**	**0.074**	**0.159**	**0.052**	**0.058**
SD	0.038	**0.202**	**0.063**	**0.173**	**0.087**	0.024	**0.153**	**0.197**	**0.108**	**0.332**	**0.091**	**0.219**	**0.176**	**0.147**	**0.319**	**0.268**	**0.126**	**0.209**	**0.319**	**0.144**	**0.379**	**0.361**	**0.401**	**0.286**	0.035	**0.135**	**0.057**	**0.063**
PO	0.045	**0.197**	0.062	**0.168**	0.091	0.018	**0.155**	**0.189**	**0.100**	**0.315**	**0.082**	**0.207**	**0.189**	**0.145**	**0.304**	**0.256**	**0.123**	**0.203**	**0.308**	**0.145**	**0.350**	**0.347**	**0.388**	**0.276**	0.042	**0.129**	0.038	**0.043**
HF	-0.020	0.069	-0.009	0.050	-0.005	-0.006	0.032	**0.085**	0.037	**0.176**	0.068	**0.089**	0.031	**0.079**	**0.168**	**0.128**	0.024	**0.079**	**0.170**	0.027	**0.227**	**0.200**	**0.235**	**0.135**	-0.021	**0.128**	**0.085**	**0.091**
CT	**0.049**	**0.204**	**0.071**	**0.174**	**0.097**	**0.031**	**0.160**	**0.194**	**0.107**	**0.323**	**0.091**	**0.216**	**0.187**	**0.145**	**0.312**	**0.263**	**0.129**	**0.209**	**0.313**	**0.150**	**0.361**	**0.353**	**0.393**	**0.282**	0.050	**0.133**	**0.060**	**0.066**
MA	**0.068**	**0.217**	**0.091**	**0.191**	**0.115**	**0.055**	**0.170**	**0.208**	**0.127**	**0.334**	**0.102**	**0.233**	**0.188**	**0.149**	**0.324**	**0.275**	**0.147**	**0.223**	**0.325**	**0.158**	**0.377**	**0.358**	**0.397**	**0.291**	**0.069**	**0.118**	**0.050**	**0.058**
MB	**0.111**	**0.214**	**0.116**	**0.194**	**0.141**	**0.100**	**0.178**	**0.211**	**0.147**	**0.312**	**0.159**	**0.224**	**0.198**	**0.166**	**0.306**	**0.262**	**0.157**	**0.219**	**0.310**	**0.162**	**0.345**	**0.334**	**0.374**	**0.277**	**0.114**	**0.169**	**0.099**	**0.109**
ST	**0.117**	**0.212**	**0.119**	**0.193**	**0.144**	**0.106**	**0.179**	**0.210**	**0.149**	**0.307**	**0.163**	**0.222**	**0.197**	**0.167**	**0.301**	**0.259**	**0.158**	**0.218**	**0.306**	**0.162**	**0.338**	**0.328**	**0.367**	**0.274**	**0.119**	**0.163**	**0.104**	**0.114**
LU	0.031	**0.176**	0.049	**0.148**	0.075	0.016	**0.134**	**0.170**	**0.088**	**0.292**	**0.087**	**0.187**	**0.161**	**0.128**	**0.281**	**0.233**	**0.105**	**0.181**	**0.284**	**0.124**	**0.328**	**0.323**	**0.363**	**0.252**	0.032	**0.142**	**0.061**	**0.066**
SB	-0.009	**0.093**	0.003	**0.075**	0.009	-0.008	0.053	**0.106**	0.048	**0.206**	0.048	**0.115**	0.054	**0.087**	**0.195**	**0.157**	0.046	**0.106**	**0.201**	0.047	**0.261**	**0.229**	**0.264**	**0.165**	-0.018	**0.072**	**0.049**	**0.054**
PT	0.046	**0.200**	0.063	0.171	0.093	0.019	**0.158**	**0.192**	**0.102**	**0.318**	**0.086**	**0.209**	**0.194**	**0.148**	**0.307**	**0.259**	**0.125**	**0.205**	**0.311**	**0.148**	**0.353**	**0.350**	**0.392**	**0.279**	0.044	**0.138**	0.040	**0.046**
YM	0.021	0.030	0.007	0.028	0.010	0.042	0.011	0.065	0.051	**0.097**	**0.117**	0.043	0.012	**0.088**	**0.092**	**0.074**	0.015	0.040	**0.103**	0.009	**0.143**	**0.113**	**0.140**	**0.073**	0.021	**0.153**	**0.129**	**0.137**
Nah	0.022	0.025	0.002	0.023	0.010	0.058	0.029	0.028	0.043	**0.018**	**0.142**	0.011	0.034	**0.088**	0.016	0.010	0.002	0.018	0.023	0.028	**0.067**	**0.028**	**0.047**	0.002	0.026	**0.184**	**0.174**	**0.183**
GT	0.017	0.018	0.011	0.018	0.013	**0.045**	0.006	**0.054**	**0.056**	**0.078**	**0.109**	0.028	0.005	**0.090**	**0.074**	**0.062**	0.008	0.030	**0.089**	0.002	**0.129**	**0.086**	**0.121**	0.052	0.027	**0.144**	**0.118**	**0.124**

Table S5b *C. intestinalis* (contined)

	CR	BR	MR	SD	PO	HF	CT	MA	MB	ST	LU	SB	PT	YM	Nah
Sho															
Shs															
Gpt															
Sth															
Lym															
Poo															
Tor															
Brx															
Ply															
Fal															
StV															
StM															
StQ															
Per															
Tre															
Blo															
AbW															
Cha															
MBl															
Cam															
Con															
Lor															
Cro															
Qui															
Grun															
GullF															
Fiske															
CR															
BR	**0.056**														
MR	0.036	0.023													
SD	-0.004	**0.072**	**0.050**												
PO	-0.006	0.006	0.006	0.002											
HF	0.032	**0.115**	**0.100**	0.018	0.054										
CT	-0.011	**0.071**	**0.046**	-0.006	-0.005	0.043									
MA	0.036	**0.087**	**0.078**	0.026	0.042	**0.068**	0.026								
MB	**0.136**	**0.199**	**0.193**	**0.129**	**0.151**	**0.139**	**0.128**	0.009							
ST	**0.147**	**0.207**	**0.202**	**0.147**	**0.161**	**0.150**	**0.142**	0.020	-0.033						
LU	-0.021	**0.102**	0.065	-0.024	-0.009	0.021	-0.022	0.032	**0.143**	**0.156**					
SB	0.011	**0.058**	**0.050**	0.018	0.025	-0.015	0.032	**0.057**	**0.114**	**0.122**	0.015				
PT	-0.007	0.035	0.003	0.001	-0.039	0.055	-0.007	0.044	**0.156**	**0.166**	-0.010	0.025			
YM	**0.121**	**0.199**	**0.192**	**0.130**	**0.142**	0.031	**0.138**	**0.116**	**0.115**	**0.115**	**0.114**	0.043	**0.145**		
Nah	**0.162**	**0.232**	**0.226**	**0.173**	**0.177**	0.041	**0.180**	**0.189**	**0.197**	**0.195**	**0.155**	0.056	**0.180**	0.009	
GT	**0.114**	**0.152**	**0.147**	**0.110**	**0.117**	0.037	**0.118**	**0.099**	**0.090**	**0.093**	**0.103**	**0.051**	**0.118**	0.009	0.008

Table S6. Estimates of pairwise population genetic differentiation for *Ciona robusta* (A) and *C. intestinalis* (B) based on concatenated mitochondrial DNA sequences.

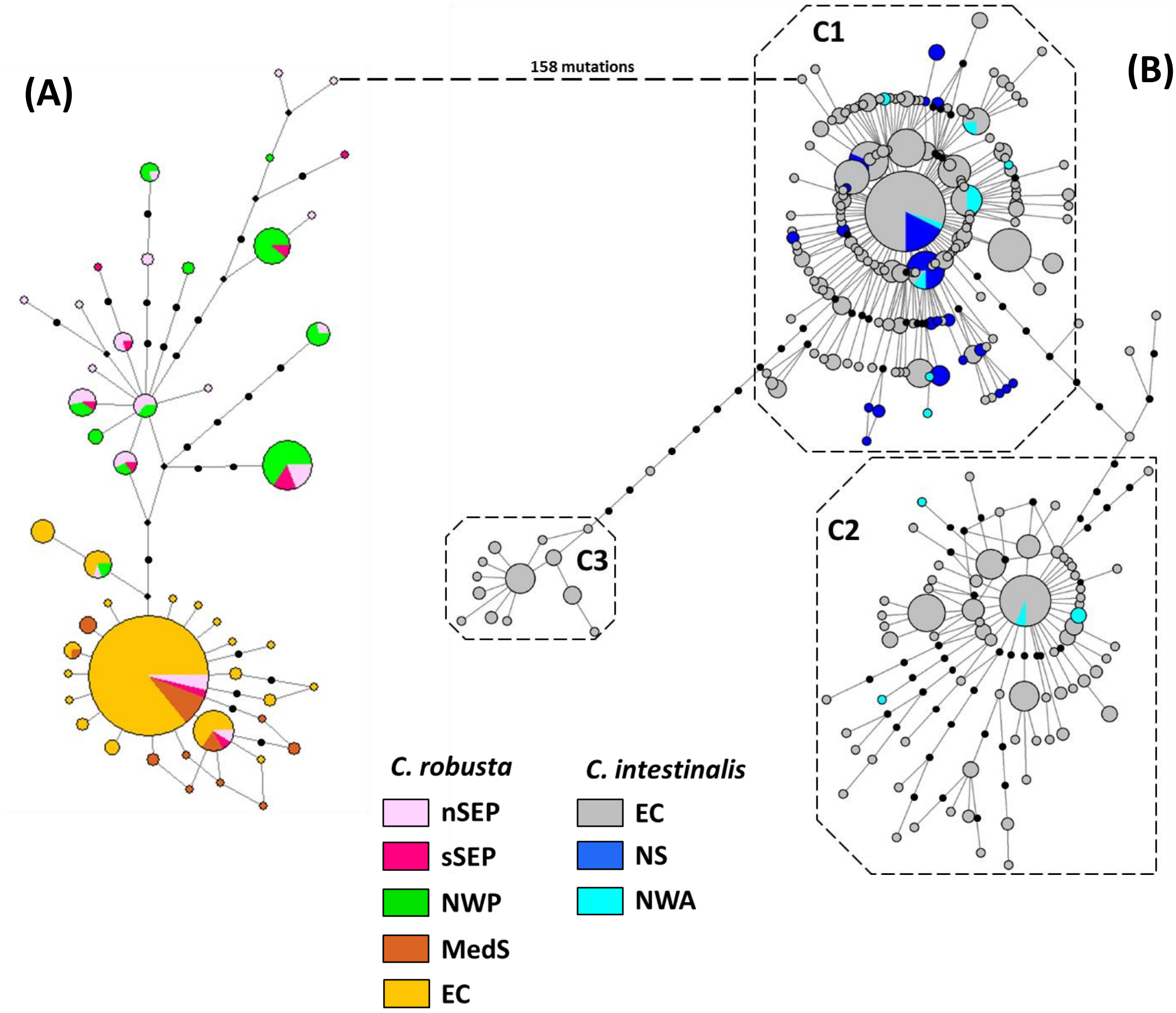
The fixation index ϕ_{ST} was computed based on concatenated mitochondrial DNA sequences (COI and COX3-ND1) with the software Arlequin v 3.5. Bold numbers indicate statistical significance (*P*-value <0.05). Population labels are detailed in Table S1.

(A) *C. robusta*

	Blo	Cha	MBl	Cam	Con	Cro	Fal	Per	Ply	StQ	StM	Tre	StV	Guana	Coqui	Nishi	Tokyo	Napl
Blo																		
Cha	0.022																	
MBl	0.003	-0.017																
Cam	0.016	0.048	-0.003															
Con	-0.029	0.045	0.023	0.043														
Cro	**0.188**	**0.288**	**0.294**	**0.348**	**0.181**													
Fal	**0.224**	**0.253**	**0.264**	**0.274**	**0.258**	**0.293**												
Per	0.002	0.023	0.000	0.000	0.022	**0.285**	**0.255**											
Ply	0.038	0.056	0.055	0.061	0.058	**0.197**	0.078	0.051										
StQ	0.040	**0.069**	**0.071**	**0.079**	**0.058**	**0.147**	**0.187**	**0.066**	**0.064**									
StM	0.014	0.045	-0.005	0.000	0.041	**0.342**	**0.269**	-0.002	0.058	**0.076**								
Tre	0.007	0.032	0.000	0.000	0.029	**0.313**	**0.264**	0.000	0.055	**0.072**	-0.002							
StV	-0.029	0.045	0.000	0.043	-0.043	**0.181**	**0.258**	0.022	0.058	**0.058**	0.041	0.029						
Guana	**0.460**	**0.494**	**0.511**	**0.507**	**0.498**	**0.487**	**0.405**	**0.499**	**0.452**	**0.437**	**0.501**	**0.503**	**0.498**					
Coqui	**0.364**	**0.399**	**0.416**	**0.412**	**0.401**	**0.391**	**0.325**	**0.404**	**0.361**	**0.349**	**0.406**	**0.408**	**0.401**	-0.014				
Nishi	**0.508**	**0.535**	**0.549**	**0.544**	**0.540**	**0.538**	**0.451**	**0.540**	**0.499**	**0.522**	**0.539**	**0.542**	**0.540**	**0.056**	**0.088**			
Tokyo	**0.575**	**0.601**	**0.615**	**0.611**	**0.606**	**0.602**	**0.509**	**0.560**	**0.562**	**0.584**	**0.606**	**0.608**	**0.606**	**0.071**	**0.122**	0.006		
Napl	0.000	0.036	0.021	0.033	-0.034	0.156	**0.244**	0.019	0.052	**0.051**	0.030	0.024	-0.034	**0.488**	**0.392**	**0.535**	**0.601**	
Sete	**0.103**	**0.151**	**0.163**	**0.171**	**0.120**	0.056	**0.214**	**0.157**	**0.137**	**0.104**	**0.166**	**0.164**	**0.120**	**0.425**	**0.338**	**0.497**	**0.555**	**0.111**

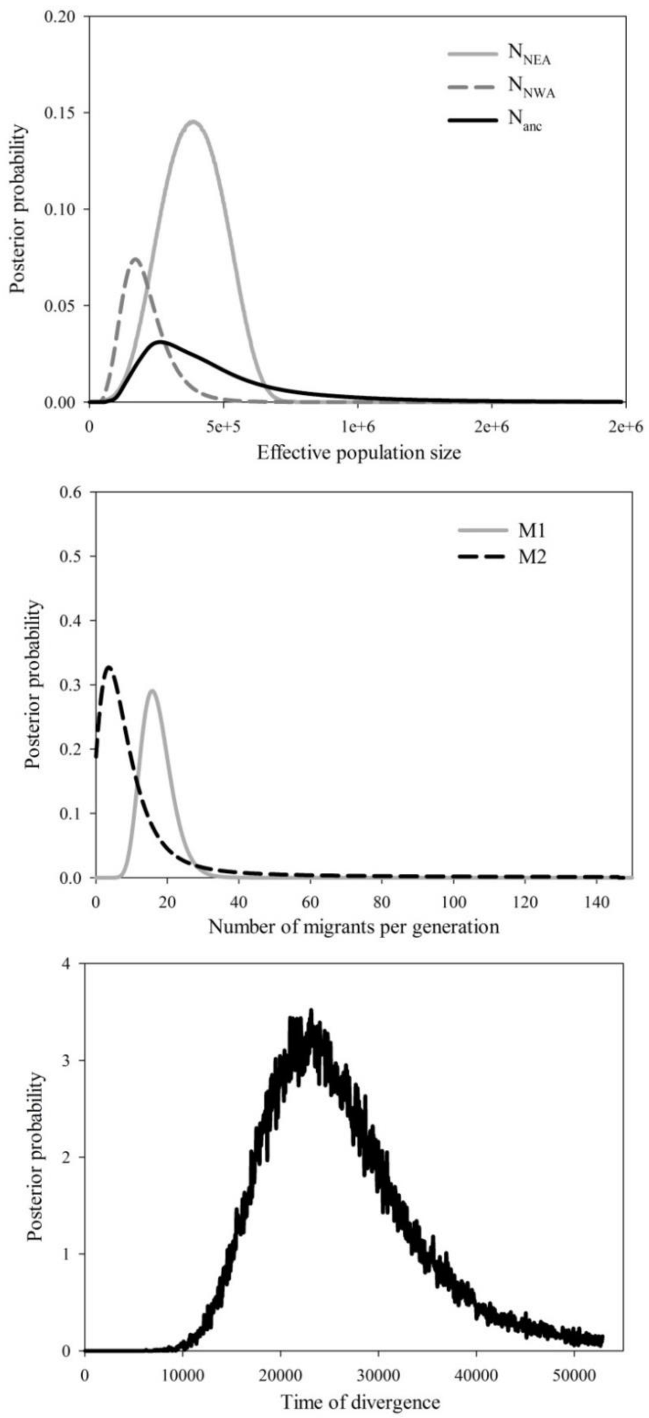
(B) *C. intestinalis*

	AbW	Blo	Cha	Bri	MBl	Brx	Cam	Con	Cro	Fal	Gpt	Lor	Lym	Per	Ply	Poo	Qui	Sho	Shs	StQ	Sth	StM	Tre	Tor	StV	Fiske	Grun	GullF
AbW																												
Blo	-0.012																											
Cha	-0.025	-0.015																										
Bri	0.052	0.002	0.048																									
MBl	0.028	-0.010	-0.021	0.050																								
Brx	0.045	0.006	0.029	0.013	0.045																							
Cam	0.016	**0.069**	0.014	**0.175**	0.027	**0.139**																						
Con	-0.009	0.036	0.000	**0.130**	-0.014	**0.117**	-0.005																					
Cro	-0.021	0.012	-0.025	**0.092**	-0.019	**0.073**	-0.012	-0.028																				
Fal	**0.162**	**0.099**	**0.190**	**0.089**	**0.182**	**0.106**	**0.312**	**0.276**	**0.228**																			
Gpt	-0.022	-0.020	-0.020	0.021	-0.026	0.024	0.050	0.009	-0.008	0.145																		
Lor	0.005	**0.064**	0.012	**0.172**	0.006	**0.145**	-0.015	-0.029	-0.020	0.316	0.034																	
Lym	**0.094**	0.035	**0.097**	0.002	**0.091**	0.037	**0.229**	**0.182**	**0.142**	**0.078**	**0.049**	**0.225**																
Nah	-0.008	-0.009	0.000	0.035	-0.008	**0.034**	**0.080**	0.027	0.011	0.132	-0.012	0.050	0.068															
Per	-0.009	0.035	0.004	**0.115**	-0.011	**0.103**	0.002	-0.027	-0.022	**0.250**	0.009	-0.020	**0.162**															
Ply	-0.009	0.029	0.006	**0.112**	-0.013	0.092	0.013	-0.027	-0.016	**0.255**	0.005	-0.015	**0.162**	-0.022														
Poo	-0.011	-0.018	-0.011	0.012	-0.017	0.027	**0.075**	0.024	0.009	**0.135**	-0.021	0.049	0.042	0.017	0.014													
Qui	0.067	0.014	0.072	-0.006	0.066	0.015	0.205	**0.154**	**0.113**	**0.078**	0.035	**0.194**	-0.001	**0.133**	**0.133**	0.022												
Sho	-0.021	-0.010	-0.015	0.044	-0.022	0.045	0.040	-0.002	-0.014	**0.165**	-0.024	0.018	**0.083**	-0.004	-0.005	-0.013	0.053											
Shs	0.036	-0.006	0.031	-0.016	0.030	0.012	**0.153**	**0.099**	0.069	**0.082**	0.005	**0.140**	-0.003	**0.088**	**0.085**	-0.003	0.000	0.018										
StQ	**0.077**	0.037	0.058	0.058	**0.083**	-0.005	**0.156**	**0.154**	**0.108**	**0.155**	**0.063**	**0.183**	**0.086**	**0.148**	**0.132**	**0.074**	**0.076**	**0.090**	**0.062**									
Sth	0.027	-0.009	0.030	-0.006	0.020	0.020	**0.144**	**0.092**	0.063	**0.099**	-0.007	**0.129**	-0.005	**0.079**	**0.077**	-0.012	-0.006	0.019	-0.019	**0.068**								
StM	-0.027	-0.030	-0.028	0.014	-0.027	0.009	0.051	0.012	-0.010	**0.147**	-0.031	0.035	**0.056**	0.009	0.004	-0.030	0.027	-0.028	-0.002	0.048	-0.007							
Tre	-0.019	0.007	-0.024	**0.086**	-0.018	0.055	-0.004	-0.011	-0.026	**0.218**	-0.007	-0.003	**0.135**	-0.011	-0.003	0.011	**0.103**	-0.003	**0.067**	**0.081**	0.055	-0.012						
Tor	0.024	0.016	0.012	0.060	0.028	0.003	**0.077**	0.061	0.032	**0.172**	0.018	**0.082**	**0.097**	0.061	0.039	0.029	**0.078**	0.024	0.047	0.005	0.050	0.002	0.024					
StV	-0.020	0.002	-0.007	0.053	-0.023	0.055	0.031	-0.008	-0.018	**0.192**	-0.018	0.011	**0.101**	-0.015	-0.013	-0.010	**0.072**	-0.021	0.033	**0.107**	0.030	-0.017	-0.008	0.038				
Fiske	**0.220**	**0.139**	**0.251**	**0.111**	**0.226**	**0.135**	**0.371**	**0.336**	**0.284**	**0.060**	**0.187**	**0.381**	**0.085**	**0.305**	**0.313**	**0.174**	**0.095**	**0.213**	**0.103**	**0.181**	**0.125**	**0.199**	**0.270**	**0.217**	**0.241**			
Grun	**0.241**	**0.178**	**0.273**	**0.178**	**0.255**	**0.181**	**0.372**	**0.349**	**0.301**	**0.098**	**0.217**	**0.387**	**0.148**	**0.315**	**0.328**	**0.213**	**0.168**	**0.240**	**0.167**	**0.223**	**0.174**	**0.232**	**0.287**	**0.244**	**0.266**	**0.091**		
GullF	**0.200**	**0.114**	**0.218**	**0.072**	**0.198**	**0.103**	**0.344**	**0.309**	**0.257**	**0.117**	**0.153**	**0.353**	0.026	**0.277**	**0.285**	**0.140**	**0.054**	**0.184**	**0.066**	**0.150**	**0.072**	**0.163**	**0.240**	**0.186**	**0.212**	**0.075**	**0.188**	
Nah	-0.008	-0.009	0.000	0.035	-0.008	**0.034**	**0.080**	0.027	0.011	0.132	-0.012	0.050	0.068	0.020	0.019	-0.013	0.026	-0.015	0.009	**0.091**	0.012	-0.020	0.014	0.037	-0.004	**0.182**	**0.204**	**0.169**



Supplementary Figure S1. Median-joining haplotype networks of *Ciona robusta* (a) and *C. intestinalis* (b) based on concatenated mtDNA sequences.

Haplotype circles are proportional to haplotype frequency in the whole dataset. Branch lengths are proportional to number of mutational steps between two haplotypes. Missing haplotypes are indicated by small black circles. Colors represent the location of individuals possessing the haplotypes. A dotted line gives the number of mutations between the most similar haplotypes in the two species.



Supplementary Figure S2. Marginal posterior distribution of parameters in the IMM computed with IMa2 for examining the history of isolation between populations of *C. intestinalis*.

Distribution curves are shown for 1) the effective size of the study populations, namely NE Atlantic and NW Atlantic (N_{NEA} and N_{NWA}, respectively) and their ancestral population (N_{anc}), 2) the number of effective migrants per generation (with M_1 and M_2 being the number of migrants from N_{NEA} to N_{NWA} and from N_{NWA} to N_{NEA}, respectively) and 3) the time of divergence between the two sides of the Atlantic. The median value and the 95% highest posterior density of each parameter are given in Figure 5 in the main text.