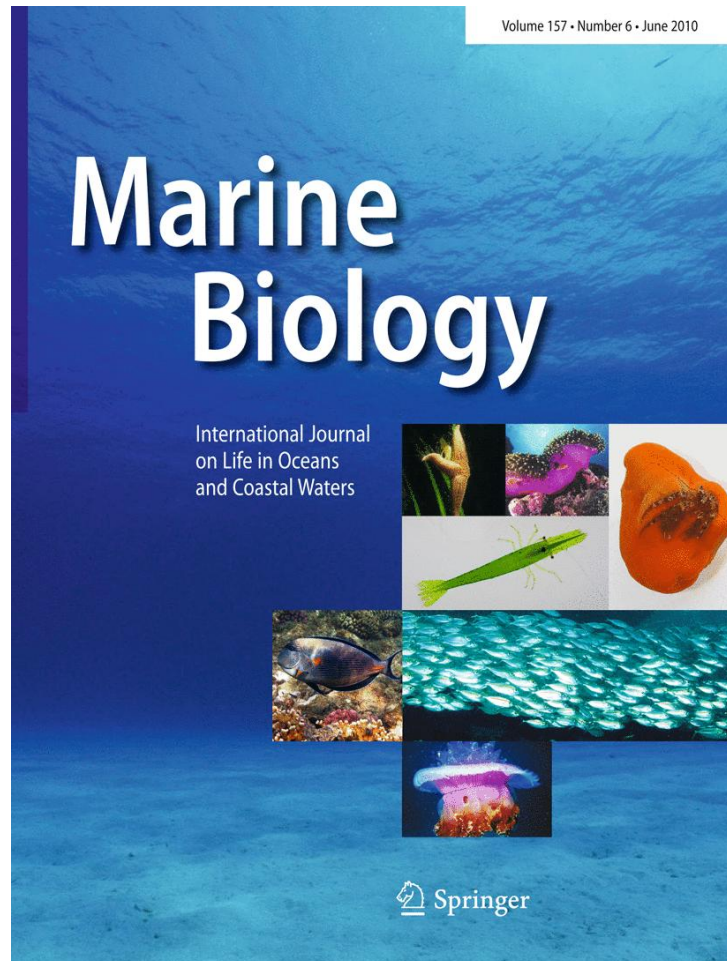


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# Phylogeography of the rock-pool copepod *Tigriopus brevicornis* (Harpacticoida) in the northern North Atlantic, and its relationship to other species of the genus

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**Abstract** We investigated relationships among North Atlantic *Tigriopus brevicornis* populations and their relationships to Mediterranean *T. fulvus* and North American *T. californicus*, using crossing experiments and mitochondrial DNA sequencing. All *T. brevicornis* populations tested were interfertile, while interspecific crosses produced either no offspring or offspring that did not survive past the larval stage, with the exception of a few *T. brevicornis* × *T. californicus* crosses that produced mature adults. DNA sequencing of a fragment of mitochondrial cytochrome oxidase I (COI) showed that samples of *T. brevicornis* from Iceland, the Faroes, Ireland, Scotland and Nova Scotia formed a single shallow clade. In contrast, *T. brevicornis* from more southern populations in France and Portugal formed a clade with substantially greater branch lengths. *Tigriopus brevicornis* was monophyletic, and *T. brevicornis* plus Mediterranean *T. fulvus* were together also monophyletic. The phylogeography of *T. brevicornis* closely mirrored that found in *T. californicus*, with substantially reduced interpopulation divergence at northern latitudes. The known distribution of *T. brevicornis* in Iceland and the Faroes is shown and dispersal mechanisms and habitat selection briefly discussed.

## Introduction

Marine species with limited dispersal capacity are useful for the study of phylogeography, offering potentially greater resolution in inferring current directions, dispersal barriers and climatic history. The copepod genus *Tigriopus* presents the counterintuitive combination of extremely restricted dispersal (e.g. Burton and Lee 1994) and wide geographic distribution (see below), making it possible to test whether the same phylogenetic patterns are repeated in distant regions. Because *Tigriopus* spp. are amenable to laboratory breeding experiments (e.g. Battaglia 1982; Edmands 1999), it also becomes possible to test whether genetic patterns are concordant with reproductive patterns.

*Tigriopus* spp. are found on rocky shores nearly worldwide. They occur, for example, both on continents and on far offshore islands such as Kerguelen, Crozet Islands and South Georgia (Davenport et al. 1997). They are found under extreme cold conditions on the Antarctic Peninsula (Waller et al. 2006), under subtropical conditions in Mexico (Ganz and Burton 1995; Edmands 2001) and in tropical conditions in Southern Asia (Jung et al. 2006; Ki et al. 2008). *Tigriopus* spp. are typically found in high shore splash-pools, although they are also known to occur subtidally on the Antarctic Peninsula (Waller et al. 2006). Also, Lang (1948) mentions a unique record of *Tigriopus brevicornis* at depth of 10 m off the coast of Sweden. In the northern hemisphere, 6 species have been recognized (Davenport et al. 1997). While the taxonomy in the southern hemisphere is less well understood, there are at least 6 species, not all of which have been described (e.g. Bradford 1967; Soyer et al. 1987; Davenport et al. 1997). To our knowledge, sympatry of two or more species has not been recorded in any location. In the northern North Atlantic, the only species found appears to be *T. brevicornis*, with a

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known distribution ranging from Portugal in the south to Iceland and Nova Scotia in the north (Ingólfsson and Steinarsdóttir 2006). Its discovery in the Faroes, Iceland and Nova Scotia is recent and quite surprising (Ingólfsson and Steinarsdóttir 2006), but in all these regions, it is common, being recorded from many localities. On the western coasts of North America, *T. brevicornis* is replaced by *T. californicus*, and in the Mediterranean by *T. fulvus*, which has also been reported from the Atlantic Islands of Madeira (Davenport et al. 1997). Both of these species differ morphologically in only minute details from *T. brevicornis*, which was only recently recognized as taxonomically distinct from *T. fulvus* (Carli and Fiori 1977).

On the western coasts of North America, it has been found that southern populations of *T. californicus*, in Mexico and California, may be quite genetically distinct from each other, while more northern populations, from Oregon to Alaska, appear to be derived, with substantially lower interpopulation divergence (Edmunds 2001). This has been attributed to a more recent origin of the northern populations, which cannot have settled in these northern regions until the ice retreated about 12–15 thousand years ago after the last glacial maximum (Edmunds 2001). The situation in the North Atlantic parallels these conditions. During the last glacial maximum, the ice shield reached as far south as Britain and France, while the offshore islands of the Faroes and Iceland were with little doubt covered by separate ice shields (e.g. Sejrup et al. 2005). Survival of *Tigriopus* spp. north of the edge of the ice shields is improbable.

Here, we examined genetic and reproductive relationships of *T. brevicornis* in the North Atlantic, using mitochondrial DNA sequencing and crossing experiments. The

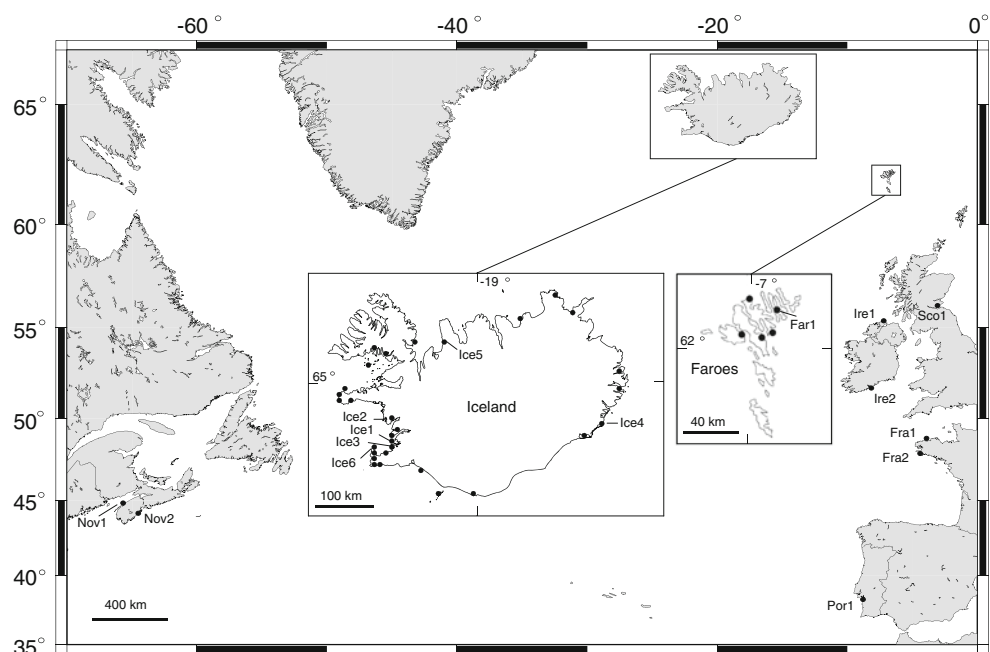
primary questions we addressed were: (1) Does the population genetic structure of *T. brevicornis* in the northern North Atlantic parallel that of *T. californicus* in the Pacific? (2) What are the genetic and reproductive relationships of *T. brevicornis* intraspecifically and to *T. californicus* and *T. fulvus*? Secondly, we also wanted to determine whether the habitat preferences of *T. brevicornis* in Iceland, where it has only recently been discovered, were similar to those elsewhere. Finally, we wanted to investigate the possible role of birds and floating algae as dispersal agents that could help explain the species' wide distribution in the face of seemingly limited dispersal capacity.

## Materials and methods

### Samples and distribution

We obtained samples from virtually the whole known distributional range of *T. brevicornis*, from Portugal in the south to Iceland and Nova Scotia in the north (Fig. 1). We also obtained samples of *T. fulvus* from the Mediterranean coast of Spain. Pacific animals were obtained from southern California. Sampling locations are shown in Table 1. Specimens were either preserved in  $\geq 96\%$  ethanol for mtDNA assays or brought alive to the laboratories of the Institute of Biology, University of Iceland or the Department of Biological Sciences, University of Southern California, Los Angeles, for crossing experiments. Cultures were held at 12°C and 12:12 d:l light regime in Iceland. In California, cultures were held at 15 or 20°C under a light regime of 12:12 d:l. The animals were acclimatized to these condi-

**Fig. 1** Labeled localities provided *Tigriopus brevicornis* samples for the current study. Additional circles in the Iceland inset indicate records of *T. brevicornis* from 2003, when it was first discovered, to 2008. Localities are grouped into 10 × 10 km squares (see Ingólfsson 1996). Additional circles in the Faroes inset indicate records of *T. brevicornis* from June 2004



**Table 1** Localities of *Tigriopus brevicornis* (TB), *T. fulvus* (TF) and *T. californicus* (TC) samples used in habitat studies, crosses and/or DNA sequencing

	Location	Habitat	Crosses	Sequenced (no. samples)	Latitude	Longitude
TB_Far1	Kunoy, Faroes			X (3)	62°17.6'	−6°40.2'
TB_Fra1	Callot, France		X	X (2)	48°43.8'	−3°59.9'
TB_Fra2	Concarneau, France			GenBank	47°52'	−3°55'
TB_Nov1	Parker's Cove, Nova Scotia		X	X (5)	44°48.8'	−65°32.2'
TB_Nov2	Peggy's Cove, Nova Scotia		X	X (1)	44°29.6'	−63°55.0'
TB_Ice1	Seltjarnarnes, Iceland	X	X		64°09.8'	−22°00.6'
TB_Ice2	Borgarnes, Iceland			X (2)	64°32.1'	−21°55.4'
TB_Ice3	Hvaleyri, Iceland			X (4)	64°03.7'	−21°59.8'
TB_Ice4	Hvalnes, Iceland			X (2)	64°24.2'	−14°32.4'
TB_Ice5	Skagaströnd, Iceland			X (1)	65°49.6'	−20°19.4'
TB_Ice6	Garðskagi, Iceland	X	X		64°05.1'	−22°41.7'
TB_Ire1	Malin Head, Ireland		X		55°22'	−7°20'
TB_Ire2	Cork, Ireland			GenBank	51°57'	−8°40'
TB_Sco1	Firth of Forth, Scotland			X (2)	55°59.9'	−3°23.9'
TB_Por1	Sines, Portugal		X	X (2)	37°57.7'	−8°55.3'
TF_Fra3	Banyuls, France			Edmands (2001)	42°28.9'	3°08.3'
TF_Spa1	Tossa de Mar, Spain		X		41°43.4'	2°56.2'
TF_Spa2	Cala de Saint Fransesc, Spain		X		41°40.7'	2°48.5'
TF_Spa3	Blanes, Spain		X	Edmands (2001)	41°40.2'	2°47.5'
TC_Ala1	Starrigavan Campground, AK, USA			Edmands (2001)	57°08'	−135°22'
TC_Ala2	Japonski Island, AK, USA			Edmands (2001)	57°03'	−135°21'
TC_Bri1	Grappler Point, BC, Canada			Edmands (2001)	48°50'	−125°08'
TC_Cal1	San Diego, CA, USA		X	Edmands (2001)	32°45'	−117°15'
TC_Cal2	Royal Palms, CA, USA		X	Edmands (2001)	33°43.0'	−118°19.2'
TC_Cal3	Carmel, CA, USA			Edmands (2001)	36°33'	−121°55'
TC_Cal4	Bodega, CA, USA			Edmands (2001)	38°19'	−123°04'
TC_Mex1	Playa Altamira South, Baja California, Mexico			Edmands (2001)	28°32'	−114°05'
TC_Ore1	Bob Creek Wayside, OR, USA			Edmands (2001)	44°15'	−124°07'
TC_Was1	Friday Harbor, WA, USA			Edmands (2001)	48°33'	−123°00'

X current study; AK Alaska; BC British Columbia; CA California; OR Oregon; WA Washington

tions for at least two months before experiments commenced, with the exception of the TB\_Nov1 × TB\_Por1 P × P crosses (both reciprocals, see Table 2), in which we acclimatized the animals for only a few days.

As *T. brevicornis* had only recently been discovered in the Faroes, Iceland, and Nova Scotia, the species was searched for in numerous localities in each region. The results from Nova Scotia have already been published (Ingólfsson and Steinarsdóttir 2006).

#### Habitats

We studied the habitats of *T. brevicornis* in detail at two localities in southwestern Iceland, Seltjarnarnes on 10 September 2004 (19 upper shore rock-pools) and Garðskagi on 2 and 3 November 2004 (42 rock-pools). The pools were

measured (height above chart datum, length, width, maximum depth) and vegetation noted if present (classified into green, brown and encrusting algae). Mean high water of springs at these localities is about 4.0 m above chart datum, and mean high water of neaps is about 3.0 m above chart datum (Anonymous 2008). Garðskagi is considerably more exposed than Seltjarnarnes.

#### Examination of floating algae and purple sandpipers (*Calidris maritima*)

We tested the hypothesis (Davenport et al. 1997) that *Enteromorpha* floating on the surface of the sea could serve as a dispersal agent for *Tigriopus* spp. We examined 6 clumps of floating algae collected in the Bay of Faxaflói, western Iceland, in July and August 1995 and 1996. All

**Table 2** Intra- and interspecific P × P crosses and controls involving *Tigriopus brevicornis* (TB), *T. fulvus* (TF) and *T. californicus* (TC)

Lab	Cross	No. replicates	Proportion replicates containing			
			Precopula	Nauplii	Copepodites	Adults
<i>Intraspecific</i>						
I	TB_Ice1 × TB_Fra1	5	1.00	0.80	0.20	0.20
I	TB_Fra1 × TB_Ice1	5	1.00	0.80	0.40	0.20
I	TB_Ice1 × TB_Por1	4	1.00	0.50	0.25	0.25
I	TB_Por1 × TB_Ice1	5	1.00	1.00	0.60	0.60
C	TB_Nov2 × TB_Ice1	10	–	0.80	0.70	0.60
I	TB_Nov1 × TB_Por1	5	1.00	1.00	1.00	1.00
I	TB_Por1 × TB_Nov1	5	1.00	1.00	1.00	1.00
<i>Interspecific TB × TF</i>						
I	TB_Ice1 × TF_Spa2	5	0.80	0.40	0.00	0.00
I	TF_Spa2 × TB_Ice1	5	0.80	0.00	0.00	0.00
I	TB_Fra1 × TF_Spa2	5	1.00	1.00	0.00	0.00
I	TF_Spa2 × TB_Fra1	5	0.20	0.00	0.00	0.00
I	TB_Por1 × TF_Spa2	5	1.00	1.00	0.00	0.00
I	TB_Spa2 × TB_Por1	5	≥0.40	0.40	0.00	0.00
C	TB_Ire1 × TF_Spa3	5	–	0.00	0.00	0.00
<i>Interspecific TB × TC</i>						
I	TB_Ice1 × TC_Cal1	5	0.20	0.00	0.00	0.00
C	TB_Ice1 × TC_Cal2**	13	–	0.46	0.31	0.31
I	TC_Cal1 × TB_Ice1**	5	0.20	0.20	0.20	0.20
C	TC_Cal2 × TB_Ice1**	7	–	0.14	0.14	0.14
C	TB_Ire1 × TC_Cal2	16	–	0.00	0.00	0.00
C	TC_Cal2 × TB_Ire1	10	–	0.00	0.00	0.00
C	TB_Nov1 × TC_Cal2	7	–	0.00	0.00	0.00
C	TC_Cal2 × TB_Nov1	6	–	0.00	0.00	0.00
C	TB_Nov2 × TC_Cal2	5	–	0.00	0.00	0.00
C	TC_Cal2 × TB_Nov2	3	–	0.00	0.00	0.00
<i>Interspecific TF × TC</i>						
C	TF_Spa1 × TC_Cal2	2	–	0.00	0.00	0.00
C	TC_Cal2 × TF_Spa1	7	–	0.00	0.00	0.00
<i>Controls TB</i>						
I	TB_Ice1 × TB_Ice1	10	≥0.80	1.00	0.70	0.50
C	TB_Ice1 × TB_Ice1	3	–	0.67	0.67	0.67
I	TB_Por1 × TB_Por1	15	≥0.80	0.60	0.53	0.53
I	TB_Fra1 × TB_Fra1	10	≥0.90	0.60	0.60	0.50
I	TB_Nov1 × TB_Nov1	5	≥0.80	0.80	0.60	0.60
C	TB_Ire1 × TB_Ire1	14	–	0.93	0.93	0.93
C	TB_Nov1 × TB_Nov1	5	–	0.80	0.40	0.40
C	TB_Nov2 × TB_Nov2	10	–	0.60	0.50	0.40
<i>Controls TF</i>						
C	TF_Spa1 × TF_Spa1	5	–	0.75	0.40	0.40
I	TF_Spa2 × TF_Spa2	5	1.00	1.00	1.00	1.00
<i>Controls TC</i>						
I	TC_Cal1 × TC_Cal1	5	≥0.80	0.80	0.60	0.60
C	TC_Cal2 × TC_Cal2	26	–	0.77	0.77	0.77

Females of each cross are listed first. Presence of pairs in precopula was not recorded (–) in experiments done in California  
 \*\* Denotes 3 crosses that unexpectedly produced adult F<sub>1</sub> offspring  
 C laboratory in California;  
 I laboratory in Iceland

**Table 3** Intra- and interspecific  $F_1 \times F_1$  crosses and controls involving *Tigriopus brevicornis* (TB), *T. fulvus* (TF) and *T. californicus* (TC)

Lab	Cross	No. replicates	Proportion replicates containing			
			Precopula	Nauplii	Copepodites	Adults
<i>Intraspecific</i>						
I	(TB_Ice1 × TB_Fra1) × (TB_Ice1 × TB_Fra1)	1	1.00	1.00	1.00	1.00
I	(TB_Fra1 × TB_Ice1) × (TB_Fra1 × TB_Ice1)	5	≥0.80	0.80	0.80	0.80
I	(TB_Ice1 × TB_Por1) × (TB_Por1 × TB_Ice1)	2	1.00	1.00	1.00	1.00
I	(TB_Por1 × TB_Ice1) × (TB_Ice1 × TB_Por1)	4	1.00	1.00	1.00	0.75
C	(TB_Nov2 × TB_Ice1) × (TB_Nov2 × TB_Ice1)	6	–	0.50	0.33	0.33
I	(TB_Nov1 × TB_Por1) × (TB_Por1 × TB_Nov1)	3	1.00	1.00	1.00	1.00
I	(TB_Por1 × TB_Nov1) × (TB_Nov1 × TB_Por1)	3	≥0.67	0.67	0.67	0.67
<i>Interspecific</i>						
C	(TB_Ice1 × TC_Cal2) × (TB_Ice1 × TC_Cal2) **	4	–	1.00	1.00	0.75
C	(TC_Cal2 × TB_Ice1) × (TC_Cal2 × TB_Ice1) **	1	–	1.00	1.00	1.00
<i>Controls</i>						
C	(TF_Spa1 × TF_Spa1) × (TF_Spa1 × TF_Spa1)	2	–	1.00	1.00	1.00
C	(TC_Cal2 × TC_Cal2) × (TC_Cal2 × TC_Cal2)	7	–	0.86	0.86	0.71

Females of each cross are always listed first. Presence of pairs in precopula was not recorded (–) in experiments done in California. \*\* Denotes 2 crosses that unexpectedly produced  $F_2$  offspring

I laboratory in Iceland; C laboratory in California

clumps contained hundreds of harpacticoids, hitherto unidentified, and substantial amounts of *Enteromorpha*-like algae, which were, however, never the chief constituents of the clumps.

The sandpiper *Calidris maritima* is another plausible dispersal agent for *Tigriopus*. The birds frequently roost on rocks at high tide and take baths in rock-pools, during which time harpacticoids may become entangled in the plumage. The birds migrate extensively, and birds ringed in Iceland have, for example, been recovered in Newfoundland (Petersen 1998). Eight birds were collected at Garðskagi at high tide on 7 September 2009, and their plumage thoroughly washed in seawater in the laboratory.

#### Crosses

We encountered unexplained difficulties in keeping cultures alive or healthy, especially in Iceland, and the slow developmental time at 12°C additionally limited the number of crosses that could be set up in the laboratory in Iceland. When possible, each cross included at least five replicates (see Table 1), although fewer replicates (2–4) were used in a small subset of crosses (4 of 38 cross types). Crosses were set up by placing precopula (male clasping a virgin female) on a piece of filter paper and then prying the animals apart under a dissecting microscope using a fine probe (see Edmands 1999 for further details). A single male and a single virgin female (copepodite stages 2–5) were then paired with new partners, one pair in each Petri dish

with filtered seawater and food (0.01 g nutritional yeast per 100 ml seawater for experiments in Iceland, 0.01 g ground *Spirulina* algae and 0.01 g ground Tetramin fish food per 100 ml filtered seawater for experiments done in California). Crosses were set up in both reciprocals (e.g. female A × male B and male A × female B), and controls were also included (e.g. A × A). Dishes were monitored for the formation of precopula and the appearance of nauplii (larvae), copepodites (juveniles) and adult copepods. In a few cases, i.e. Fra × Ice ( $F_1$ ), and the reverse, the individuals were allowed to pair on their own. The  $F_1$  offspring from parental populations (P) (whether “forcefully” paired or not), if produced, were then paired with other  $F_1$  offspring to check for fertility. A subset of these crosses were accompanied with controls (see Table 3). Insufficient numbers prevented controls for all crosses, but as most crosses produced mature adults, this was not deemed critical. Also, the controls used in P × P crosses are indicative of the outcome of conspecific crosses. In intraspecific crosses of *T. brevicornis* populations, we emphasized crossing populations that were geographically distant from each other.

#### DNA analyses

Copepods were rinsed with diH<sub>2</sub>O and placed in individual strip tubes containing 50 µl 200 µg proteinase K/ml lysis buffer. DNA was extracted by incubating samples for one hour at 65°C followed by 15 min at 100°C. PCR was used to amplify a fragment of mitochondrial cytochrome oxidase

I (COI) from 1 to 5 individuals from each location (Table 1). Overlapping fragments were amplified using one of two primer sets: (1) “universal” primers LCO1490 and HCO2198 from Folmer et al. (1994) that amplify a 710 bp fragment, or (2) custom primers TBREV-left (5'-TG TTGATAGCTCCAGCGAGA-3') and TBREV-right (5'- ATTCATTGGATCCGCCATAA-3') that amplify a 542 bp fragment. Amplification followed standard procedures (Saiki et al. 1988) using a reaction volume of 25  $\mu$ l, a MgCl<sub>2</sub> concentration of 2 mM, and an annealing temperature of 45°C. PCR products were visualized by gel electrophoresis, purified using Qiagen spin columns and sequenced at a commercial facility (University of Washington High-Throughput Genomics Unit in Seattle, WA or Geneway Research in Hayward, CA) using primers HCO2198, LCO1490, TBREV-right and/or COIVL-TBint (5'- AGGACTATGGTCGGGATTCA -3'). DNA sequences were aligned and edited using Sequencher 4.6 (Gene Codes Corporation), and PAUP\* (ver 4.01b10, Swofford 2003) was used to reconstruct phylogenetic trees. Sequences for 24 *T. brevicornis* individuals (GenBank accession numbers FJ899709-FJ899732) were compared to congeneric sequences from Edmands (2001) and from GenBank, with the calanoid *Calanus pacificus* used as an outgroup. Phylogeny reconstruction was based on a 266 bp aligned region corresponding to positions 245–496 in the *T. californicus* COI sequence (Lee 1993, GenBank accession number L43049). Trees were constructed by maximum parsimony using a branch and bound search. Pairwise distances between sequences were calculated as uncorrected (“p”) distances.

## Results

### Distribution in Iceland and the Faroes

Two of the present authors (AI, MBS) discovered *Tigriopus brevicornis* for the first time in the Faroe Islands, Iceland and Nova Scotia, thus extending the known distribution of the species considerably. The species proved to be common in all areas. It was found on four different islands in the Faroes and in numerous localities scattered around Iceland (Fig. 1). Some findings are from isolated rocky outcrops (including islands) that may be more than 50 km apart or from the closest locality suitable for *Tigriopus* spp. For distribution in Nova Scotia, see Ingólfsson and Steinarsdóttir (2006).

### Habitats

At Seltjarnarnes, the total height range of 19 pools examined was 2.72–3.75 m above chart datum. *Tigriopus brevi-*

*cornis* occurred in 8 pools, all in the range of 3.02–3.50 m above chart datum. Four pools in this range did not contain *Tigriopus*. At Garðskagi, the total range of 42 pools examined was 2.60–3.62 m. *Tigriopus* occurred in 14 pools. Their height range was 3.34–3.62 m above chart datum. All but one of the 15 pools in this height range contained *Tigriopus*. Only 2 out of the 22 pools containing *Tigriopus* examined here did not contain conspicuous green algae (*Enteromorpha* and probably others). General observations of *Tigriopus*-containing pools around the coasts of Iceland, as well as in other localities sampled, strongly indicate an association between the harpacticoid and green algae. All *Tigriopus*-containing pools were shallow, usually less than 15 cm in maximal depth.

The *Tigriopus*-containing pools at Seltjarnarnes lie between mean high water of neaps and mean high water of springs. At the more exposed Garðskagi, they lie somewhat higher, although below mean high water of springs.

### Examination of floating algae and purple sandpipers

No *Tigriopus* spp. were found in the examination of 6 clumps of seaweed containing both numerous harpacticoids and considerable amounts of *Enteromorpha* or *Enteromorpha*-like algae. No *Tigriopus* spp. or other harpacticoids were obtained from the plumage of the 8 sandpipers examined.

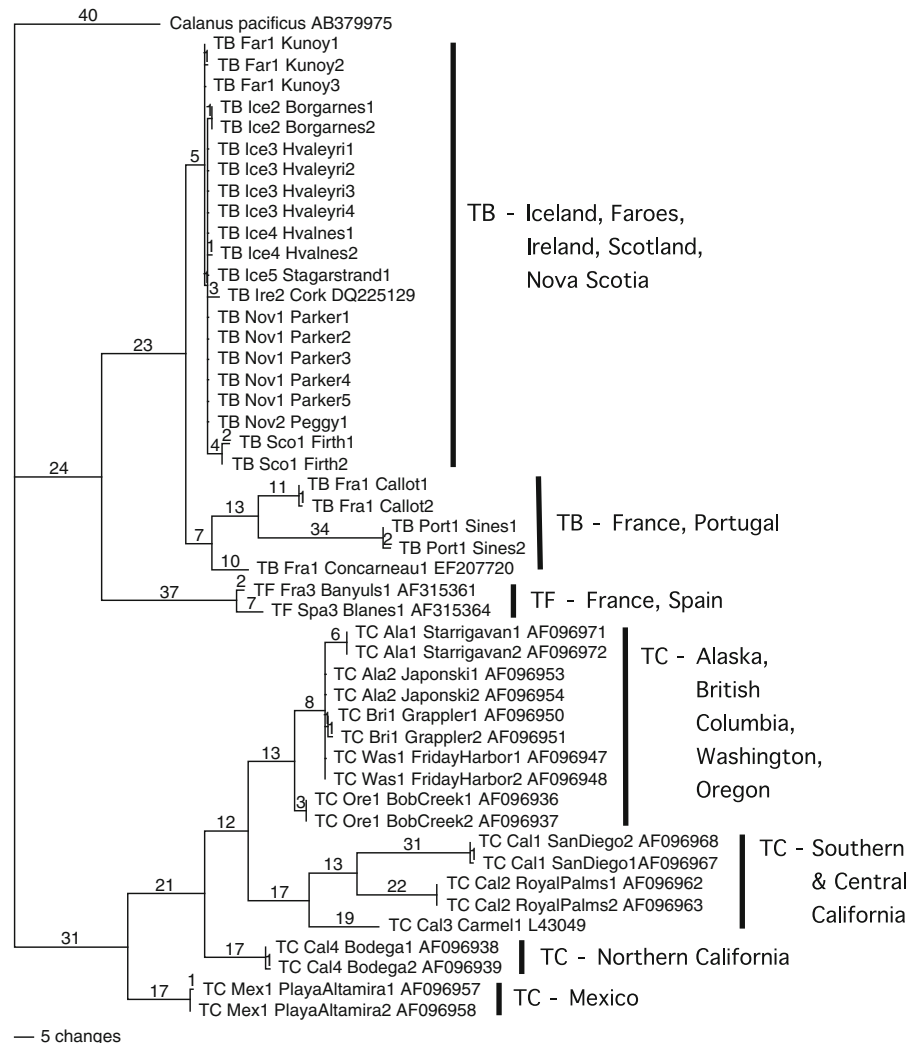
### Crosses

All P  $\times$  P intraspecific crosses attempted produced offspring that reached maturity in at least one replicate (Table 2), with no trend apparent when intra- and interpopulation crosses were compared. Thus, for *T. brevicornis*, 58% of 72 intrapopulation replicates (controls) produced adults, whereas 56% of 39 interpopulation replicates did so.

In interspecific P  $\times$  P crosses, 4 out of 7 *T. brevicornis*  $\times$  *T. fulvus* crosses produced nauplii in at least one replicate, which, however, did not develop further (Table 2). Three of 4 *T. brevicornis*  $\times$  *T. californicus* crosses between Iceland and California populations produced nauplii, which developed into adults in 6 of 30 replicates (Table 2). However, in 6 other *T. brevicornis*  $\times$  *T. californicus* crosses (altogether 47 replicates), no nauplii were produced and no *T. fulvus*  $\times$  *T. californicus* crosses (9 replicates) produced offspring. When checked, precopula were formed in 20–100% of replicates when *T. brevicornis* and *T. fulvus* were placed together, and in 20% in case of *T. brevicornis* and *T. californicus*. Offspring in some replicates of all control setups reached adulthood.

In F<sub>1</sub>  $\times$  F<sub>1</sub> intraspecific crosses, all produced mature individuals in at least one dish (Table 3). On the whole, the proportion of replicates in F<sub>1</sub>  $\times$  F<sub>1</sub> intraspecific crosses

**Fig. 2** Phylogeny constructed with maximum parsimony using a 266-base segment of mitochondrial cytochrome oxidase I, with *Calanus pacificus* used as an outgroup. The tree shown is the single most parsimonious cladogram and has a length of 464 and a consistency index of 0.550. Numbers along branches indicate the absolute number of nucleotide substitutions. Accession numbers are given for sequences obtained from GenBank. See Table 1 for locality information



producing adults, 71% of 24, was not statistically different from the results of  $P \times P$  crosses listed above (Pearson chi square = 0.47,  $df = 1$ ,  $P > 0.05$ ).

#### DNA analyses

Phylogenetic analysis of the 266 base pair region revealed 129 parsimony-informative characters resulting in a single most parsimonious tree of length 464 and consistency index 0.550 (Fig. 2). *Tigriopus brevicornis* samples from Iceland, the Faroes, Ireland, Scotland and Nova Scotia formed a single shallow clade, with a maximum pairwise sequence divergence of only 3.6% over more than 21° of latitude. *T. brevicornis* samples from more southerly regions in France and Portugal formed a clade showing substantially greater branch lengths, with up to 21.3% divergence over less than 11° latitude. Together, the *T. brevicornis* sequences formed a monophyletic group. *T. brevicornis* and *T. fulvus* together also formed a monophyletic group.

Samples of *T. californicus* from the Pacific formed a separate clade, with phylogenetic patterns consistent with a previous study (Edmands 2001), which used a larger segment (552 bp) of the same COI sequences. As with *T. brevicornis*, *T. californicus* samples from northern regions (Alaska, British Columbia, Washington and Oregon) formed a tight clade, with a maximum of 6.7% nucleotide divergence over nearly 13° latitude. In contrast, samples from California and Mexico showed more than 26.3% sequence divergence over less than 10° latitude.

#### Discussion

##### Habitat, distribution and dispersal

*Tigriopus brevicornis* is found in Iceland under similar conditions as described for the species elsewhere (e.g. Davenport et al. 1997), i.e. in rock-pools in the uppermost intertidal. It is, however, not confined to pools above the



level of mean high water of springs, as stated by McAllen (2001), being frequently found lower than this in Iceland.

The abundance of *T. brevicornis* in Iceland, the Faroe Islands and Nova Scotia indicates a relatively long history (hundreds or thousands of years) of the species in these regions, although the first discoveries are recent, 2003–2005. These populations occur in areas that were covered with ice during the last glacial maximum, and must have been settled within the last 12,000–15,000 years. In addition, it seems clear that these populations have all originated from the mainland of Europe, as there was no suitable habitat (rocky shores) south of the ice shield during the last glacial maximum (Ingólfsson 1992, 2009). It has, however, been suggested that ice-free coastal refugia may have existed in these areas, where marine species could have survived the last glacial maximum (Maggs et al. 2008). This is, however, extremely unlikely, at least when the Faroes and Iceland are concerned (Ingólfsson 2009). It is noteworthy that the species has not been recorded south of Nova Scotia on the eastern seaboard of North America, although conditions, in New England, for example, would seem ideal for the species. Together, the distribution patterns suggest occasional long-distance dispersal, a phenomenon that is also indicated by the presence of *Tigriopus* spp. on isolated islands in the southern hemisphere (Battaglia et al. 1985). DNA data, on the other hand, often indicate extremely limited dispersal (see below).

The dispersal mechanism of *Tigriopus* spp. is unknown, and to our knowledge, exclusive of Lang's account for collection at depth (1948), the animals have never been recorded from plankton samples. Davenport et al. (1997) suggest they may be carried on the surface of the oceans by floating *Enteromorpha*, as they are especially common in pools where these algae are common which is supported by our findings (see also McAllen 1999). Although our limited study of floating algal clumps did not reveal *Tigriopus* spp., this mechanism of dispersal cannot be excluded, although Powlik (1999) regards it as rather unlikely when discussing short-distance dispersal. Powlik further mentions wind and birds as dispersal agents. Our examination of purple sandpipers produced negative results, and to our knowledge, *Tigriopus* spp. has never been found on birds. However, living amphipods, which may tolerate some desiccation (although not to the same degree as *Tigriopus* spp.), have been reported from the plumage of birds (Swanson 1984). Our impression is that *Tigriopus* spp. are especially abundant in areas used by birds to roost, but this may be due to bird droppings fertilizing the pools. Another possibility might be dispersal due to use of *Tigriopus* in aquaculture as food for larval fish and crustaceans. However, we are not aware of its use as such anywhere, although mass cultivation has been done of both *T. fulvus* (Carli et al. 1995) and *T. brevicornis* (Vilela 1992) with the aim of investigating

their potential in aquaculture. While dispersal by man certainly cannot be ruled out, there are cases of long-distance dispersal of *Tigriopus* spp. that do not appear to involve human intervention, such as the far offshore southern islands of Crozet and Kerguelen where well-differentiated species have evolved (Soyer et al. 1987) indicating colonization long before man initially visited these islands. Although sub tidal records of *Tigriopus* spp. have been noted (Lang 1948; Waller et al. 2006), we consider a “bottom route” highly unlikely as a means of dispersal.

#### Crosses

We were able to confirm that the following *T. brevicornis* crosses produced fertile offspring of both sexes (females of crosses listed first): Iceland × France, France × Iceland, Iceland × Portugal, Portugal × Iceland, Portugal × Nova Scotia, Nova Scotia × Portugal. Thus, sex of the F<sub>1</sub> animals did not seem to matter and neither did geographic distance between populations.

The interspecific crosses between *T. brevicornis* and *T. fulvus* produced either no offspring or, quite often, nauplii that did not develop beyond this stage (although a few nauplii were seen to molt). This occurred both in crosses between distant populations (e.g. *T. brevicornis* from Iceland × *T. fulvus* from Spain) as well as between the neighboring *T. fulvus* from Spain and *T. brevicornis* from Portugal, indicating well-differentiated species. These results are quite similar to those obtained by Battaglia (1982), who found that crossing *Tigriopus* spp. from the Mediterranean and Atlantic populations of *Tigriopus* produced either no offspring or nauplii that died at an early age.

Results of interspecific crosses between Californian *T. californicus* and Icelandic *T. brevicornis* (F<sub>1</sub> and F<sub>2</sub> adults in both reciprocal crosses) were totally unexpected given the enormous genetic divergence between these two taxa. Attempts to genotype the putative hybrids with microsatellite markers developed for *T. californicus* (Harrison et al. 2004) were inconclusive because the loci did not generally amplify in *T. brevicornis*. One explanation for the unexpectedly successful hybridization is that putative virgin females may already have been fertilized by males from their own population. A recent study by one of us (Wetkowski and Edmands unpublished data) showed males mistakenly clasping fertilized females 0.5–3.8% of the time, depending on the availability of females. Alternatively, the two species may indeed be partially compatible, consistent with the well-established pattern of very weak correlation between genetic distance and reproductive compatibility (e.g. Edmands 2002). Consistent with this possibility is the observation that in one cross performed in Iceland where a hybrid became mature its Californian mother was seen to

molt after the pair was placed together, strongly indicating virginity. More definitive determination of reproductive compatibility between *T. californicus* and Icelandic *T. brevicornis* will require further experiments in which putative hybrids are genotyped with diagnostic markers.

#### DNA analyses

The high level of resolution found for such a small DNA fragment in a relatively small number of individuals is not typical of phylogenetic studies, but is not surprising for *Tigriopus* spp. where intrapopulation variation tends to be very low and interpopulation differentiation, particularly for mitochondrial sequences, tends to be extremely high (e.g. Edmands 2001; Willett and Burton 2004). Applying a standard molecular clock to *T. californicus* mitochondrial data would mean that populations as little as 10 km apart would have remained genetically isolated over approximately 6–8 million years (Edmands 2001) despite enormous fluctuations in sea level. The improbability of such a scenario raises the possibility that *Tigriopus* spp. have elevated mutation rates, perhaps caused by faulty proofreading enzymes, but this has not yet been tested.

Phylogeographic patterns found in northern North Atlantic *T. brevicornis* bear some similarity to those recently reported for more southerly populations of the same species. Denis et al. (2009) used the more rapidly evolving nuclear ITS1 region to compare Atlantic populations ranging from Spain to Ireland. Results showed a southern clade (42–45° latitude) with genetically isolated populations and a northern clade (46–54° latitude) with more genetically cohesive populations, patterns that were attributed to both post-glacial expansion and greater habitat continuity in the North.

Our mitochondrial phylogeny shows that both *T. californicus* and *T. brevicornis* have substantially reduced interpopulation divergence in northern regions, although the pattern is particularly extreme in *T. brevicornis* where northern populations differ by only 0.2% per latitudinal degree. Furthermore, *T. brevicornis* in Nova Scotia are identical to some individuals across the Atlantic in Iceland, a distance of over 40 longitudinal degrees. Close genetic relationships of populations across the northern North Atlantic have been found in a number of other rocky shore invertebrate species (Wares and Cunningham 2001), most likely the consequence of recent colonization of Iceland and the eastern seaboard of North America from Europe (cf. above).

Phylogeographic patterns in *Tigriopus* spp. present a conundrum. On the one hand, genetic data suggest extremely limited dispersal. Levels of interpopulation mitochondrial DNA divergence in *T. californicus* (>23%) are among the highest ever reported for conspecific populations

(Burton and Lee 1993; Edmands 2001) and these differences are temporally stable (Burton 1997). Genetic data also suggest limited effective dispersal in *T. fulvus* (Battaglia 1982), *T. japonicus* (Jung et al. 2006; Ki et al. 2008), *Tigriopus* in the southern Kerguelen Islands (Battaglia et al. 1985) and in southern populations of *T. brevicornis* (present study). On the other hand, shallow clades spanning large geographic areas in the North Pacific (*T. californicus*) and especially in the North Atlantic (*T. brevicornis*) show that *Tigriopus* species are capable of long-distance dispersal events.

#### Conclusions

The recent discovery of *T. brevicornis* in Iceland, the Faroes and Nova Scotia prompted investigations into genetic and reproductive relationships within the species, as well as to its congeners *T. californicus* and *T. fulvus*. Crossing experiments generally showed the three nominal taxa to be good biological species, as crosses between intraspecific populations showed complete interfertility, while crosses between interspecific populations produced either no offspring or offspring that failed to develop beyond the naupliar stage. Further work is needed to confirm the one curious exception to this pattern, in which a subset of crosses between *T. californicus* and Icelandic *T. brevicornis* produced mature F<sub>1</sub> and F<sub>2</sub> hybrids. Analysis of a segment of the mitochondrial COI gene also showed *T. brevicornis*, *T. fulvus* and *T. californicus* to be good phylogenetic species, as each formed a monophyletic group. Phylogenetic analyses within *T. brevicornis* revealed extensive population divergence in the south, while northern regions covered by ice during the last glacial maximum exhibited a 11-fold reduction in percent sequence divergence per latitudinal degree. This pattern is similar to but more extreme than the north–south differences previously reported for *T. californicus*. Despite extensive genetic evidence for restricted dispersal, *Tigriopus* species appear capable of occasional long-distance transport, apparently colonizing large geographic areas within the last 12–15 thousand years, and even traversing the Atlantic. The pattern is reminiscent of the ‘paradox of Rockall’ noted by Johannesson (1988), in which species with limited dispersal may be more widespread than those with extensive plankton dispersal, because restricted dispersal helps maintaining populations at sufficiently high density after a rare, long-distance transport event.

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