ULB UNIVERSITÉ LIBRE DE BRUXELLES

DI·fusion

Van De Putte, Anton, Janko, Karel, Kašparová, Eva, Maes, Grégory, Rock, Jennifer, Koubbi, Philippe, Volckaert, Filip, Choleva, Lukáš, Fraser, Keiron, Smykla, Jerzy, Van HOUDT, Jeroen K.J., & Marshall, Craig

Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species

Article. Version publiée - Published version.
© ELSEVIER

Citation APA:

Van De Putte, A., Janko, K., Kašparová, E., Maes, G., Rock, J., Koubbi, P., Volckaert, F., Choleva, L., Fraser, K., Smykla, J., Van HOUDT, J. K., & Marshall, C. (2012). Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species. *Marine Genomics*, 8, 23-34. doi:10.1016/j.margen.2012.05.002

DOI: 10.1016/j.margen.2012.05.002

Also available at: http://hdl.handle.net/2013/ULB-DIPOT:oai:dipot.ulb.ac.be:2013/337830

Cet article publié par ELSEVIER provient du Dépôt institutionnel de l'Université Libre de Bruxelles, DI-fusion http://difusion.ulb.ac.be. Il n'est accessible qu'aux membres de la communauté universitaire de l'ULB sur le réseau sécurisé de l'ULB.

Tout utilisateur autorisé peut lire, télécharger ou reproduire cet article à des fins d'usage privé ou à des fins non commerciales d'enseignement ou de recherche scientifique. Il ne peut être atteint à l'intégrité de l'article, et les noms des auteurs et de l'éditeur doivent être conservés. Tout téléchargement systématique des articles publiés par ELSEVIER mis à disposition dans DI-fusion est interdit.

This article published by ELSEVIER comes from the Institutional repository of Université Libre de Bruxelles, DI-fusion http://difusion.ulb.ac.be. It is accessible only to the members of the university community of ULB on the ULB secure network.

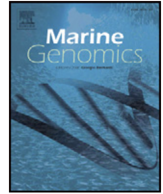
Any authorized user may read, download or reproduce this article for private usage, or for non commercial research or educational purposes. The integrity of the article and identification of the author and copyright owner must be preserved. Systematic downloading of articles published by ELSEVIER that are available in DI-fusion is not permitted.

Marine Genomics 8 (2012) 23–34

Contents lists available at SciVerse ScienceDirect

Marine Genomics

journal homepage: www.elsevier.com/locate/margen



Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species

Anton P. Van de Putte [a,b,*,1], Karel Janko [c,1], Eva Kasparova [c], Gregory E. Maes [a], Jennifer Rock [d], Philippe Koubbi [e], Filip A.M. Volckaert [a], Lukáš Choleva [c], Keiron P.P. Fraser [f], Jerzy Smykla [g,h], Jeroen K.J. Van Houdt [i], Craig Marshall [j]

[a] *Laboratory of Biodiversity and Evolutionary Genomics, KU Leuven, Ch. Deberiotstraat 32, B-3000 Leuven, Belgium*
[b] *Royal Belgian Institute for Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium*
[c] *Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Rumburská 89, 27721 Liběchov, Czech Republic*
[d] *Department of Zoology, University of Otago, Dunedin, 9054, New Zealand*
[e] *UPMC Université Paris 06, UMR 7093, Laboratoire d'Océanographie de Villefranche, BP28, 06234 Villefranche-sur-Mer, France*
[f] *Government of South Georgia and the South Sandwich Islands, Government House, Stanley, Falkland Islands*
[g] *Department of Biodiversity, Institute of Nature Conservation, Polish Academy of Sciences, Mickiewicza 33, 31–120 Kraków, Poland*
[h] *Department of Biology and Marine Biology, University of North Carolina Wilmington, 601 S. College Rd., Wilmington, NC 28403–5915, USA*
[i] *Laboratory for Cytogenetics and Genome Research, KU Leuven, O&N I Herestraat 49 - box 602 , B-3000 Leuven, Belgium*
[j] *Department of Biochemistry and Genetics Otago, University of Otago, PO Box 56, Dunedin 9054, New Zealand*

A R T I C L E I N F O

Article history:
Received 2 February 2012
Received in revised form 18 May 2012
Accepted 22 May 2012

Keywords:
Microsatellite markers
Mitochondrial
Larval duration
Glaciations
Notothenioidei
Life history

A B S T R A C T

Population genetics patterns of marine fish in general and of Southern Ocean fish in particular range from virtual panmixia over ocean-wide scale to deeply fragmented populations. However the causes underlying these different patterns are not properly understood. In this paper, we tested the hypotheses that population connectivity is positively related to a combination of life history traits, namely duration of pelagic larval period and the tendency towards pelagic life style in the adulthood. To do so, we analysed the variability of six microsatellite and one mitochondrial marker (cytochrome *b*) in three Southern Ocean fish species (*Trematomus newnesi*, *Trematomus hansoni* and *Trematomus bernacchii*). They share a recent common ancestor but notably differ in their duration of pelagic larval period as well as pelagic versus benthic lifestyle. We sampled over a range of more than 5000 km for all three species and used a number of population genetics tools to investigate past and contemporary levels of connectivity. All species experienced population fluctuations, but coalescent simulations suggested that contemporary populations are in migration-drift equilibrium. Although global F_{ST} values were rather low, a significant population structure separated the High-Antarctic from the Peninsular regions in all species. The level of genetic differentiation was much lower in the pelagic *versus* benthic species. Present data suggest that past and present genetic structuring in the Southern Ocean are indeed related with the ecological traits of Antarctic fish, however the relative importance of individual factors remains unclear.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Many marine species disperse primarily during the larval phase, while adults show limited movements (Bonhomme and Planes, 2000; Thorrold et al., 2007). Indeed, seventy percent of marine organisms have a planktonic stage during which larvae may disperse (Thorson, 1950). In general, fishes with longer larval duration display less genetic differentiation than those with shorter larval duration (Waples, 1987; Bay et al., 2006; Bradbury et al., 2008; Hauser and Carvalho, 2008). However, a growing body of evidence suggests the importance of other factors, such as currents and larval retention (Carreras-Carbonell et al., 2006), that may cause strong differentiation even in species with a long larval phase (Taylor and Hellberg, 2003; Planes et al., 1998). Many larvae are also capable of active vertical migration, which in combination with vertically stratified flows may allow them to avoid advection. In many cases, particularly in later stages of their pelagic developmental phase, larvae may be able to swim even faster than ambient currents (Leis, 2006).

Direct measurements of larval/individual dispersal of small organisms are difficult to obtain in any marine environment (Bay et al., 2006; Hedgecock et al., 2007; and references therein) but population genetics studies, although not without limitations, provide some of the most valuable tools for estimating such parameters (Hedgecock et al., 2007; Selkoe et al., 2008; Skillings et al., 2011). Populations evolve through changes in allele frequencies, which in turn change through

* Corresponding author at: Royal Belgian Institute for Natural Sciences, Vautierstraat 29, B-1000 Brussels, Belgium. Tel.: +32 2 6294318.
E-mail address: anton.vandeputte@naturalsciences.be (A.P. Van de Putte).
[1] Made an equal contribution.

1874-7787/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.margen.2012.05.002

evolutionary forces such as mutation, random genetic drift, gene flow and selection. In the absence of environmental change and over prolonged periods of time, a population will reach a genetic equilibrium determined by the opposing evolutionary forces of drift and migration. Historical factors such as climate associated extinctions and recolonisations or changes in the direction of currents also play an important role in shaping the population structure of marine species (Maggs et al., 2008). Genetic methods for estimating effective dispersal and connectivity are becoming increasingly effective. However publications often focus on low latitude reef species and general conclusions concerning the processes driving differentiation within species remain hard to draw (Bohonak, 1999; Bay et al., 2006).

The Southern Ocean, in spite of obvious challenges for sampling, offers a unique environment to study the effect of biological and environmental forces on patterns of dispersal and evolution in marine organisms. Characterised by two major currents that promote dispersal on a circumpolar scale, the majority of the surface flow moves in a clockwise (eastward) motion around Antarctica forming the Antarctic Circumpolar Current (ACC). Along the margins of the continent, however, there is a westward current, the Antarctic Slope Current (ASC) (Orsi et al., 1995). Application of genetic tools has revealed that patterns of population differentiation in Antarctic/Southern Ocean organisms range from virtual panmixia on a circum-Antarctic scale to isolated populations or even crypic species (for an overview see Rogers, 2007, 2012; Volckaert et al., 2012). The causes for such differences are not fully clear but the ultimate outcome likely depends on the interplay of several factors. Some studies emphasize the dominant roles of major climatic and geological events affecting southern polar organisms more or less simultaneously (Thatje et al., 2008). Other studies suggest that conditions for survival and dispersal differ across Antarctic biogeographic regions and hence the genetic structure of given species depends on its distribution range and the local conditions, especially temperature (McGaughran et al., 2010; Patarnello et al., 2011).

Yet other studies highlight the dominant role of intrinsic factors such as habitat preference in shaping the genetic structure of Antarctic species. Comparative analysis of four trematomids, *Trematomus bernacchi*, *Trematomus pennelli*, *Pagothenia borchgrevinki* and *Trematomus newnesi* showed a qualitatively different response of benthic and pelagic species to the Pleistocene glaciations (Janko et al., 2007). Benthic species in particular are expected to express a more pronounced genetic structure than pelagic ones (Rogers, 2007, 2012), which may either reflect lower dispersal at larval and adult stages or a higher complexity of the benthic environment, promoting habitat specialization. Such a situation may become extreme when populations have been historically separated during cycles of glaciation, suggesting that life style may decisively affect the evolution and adaptability of the species. Although Matschiner et al. (2009) recently showed that passive larval dispersal within Antarctic currents may be sufficient to homogenise populations of benthic fish species at a scale of hundreds of kilometres, one may expect that the length of pelagic larval phase in combination with specialisation to a benthic or pelagic life history in adulthood may have a prominent effect on the genetic build-up of a species' genepool (Purcell et al., 2006; Bradbury et al., 2008; Papetti et al., 2012).

The strength of a comparative approach lies in the possibility to disentangle the major determinants of population structure of Southern Ocean organisms and to broaden our scope of understanding of marine ecological speciation in general. Here we focus on the Trematominae, an endemic tribe of notothenioid teleosts from the Southern Ocean widely used as a model of cold adaptation (Patarnello et al., 2011). The group forms a monophyletic species flock strikingly diverse in morphology and demonstrates significant evolutionary versatility with a variety of ecological forms including species with benthic, pelagic or cryopelagic ecology (Janko et al., 2011; Rutschmann et al., 2011).

This study aims at comparing genetic diversity, genetic differentiation and environmental and life-history (traits) between three fish species with distinct characteristics living over the same environmental gradient. For this the nuclear and mitochondrial genetic structure and demographic history of *T. bernacchii*, *T. hansoni* and *T. newnesi* was compared. The former two are bottom-feeding fish that lay demersal eggs (with *T. bernacchii* even laying eggs in sponges and displaying nest guarding behaviour) (DeWitt et al., 1990; Kock et al., 2006; La Mesa et al., 2006). In contrast, *T. newnesi* prefers a semi-pelagic or even cryopelagic habitat. The larval phase of all three species is pelagic but differs in duration; *T. hansoni* has a long larval phase spanning over the winter, while the pelagic larval phase of *T. newnesi* is considerably shorter and accomplished during the first fall (Duhamel et al., 1993; detailed information on larval ecology of *T. bernacchii* remains undetermined). Adult individuals may differ substantially in dispersal ecology, since *T. newnesi* populations are known to largely vary in local abundance, indicative of seasonal migrations, while *T. bernacchii* is a rather sedentary fish (as an extreme example of this behaviour, we noted the re-capture of a specimen at the Ross Sea region, which had been tagged two years ago in the very same spot (J. MacDonald pers. Comm..). We hypothesize that life history traits such as larval duration and adult ecology affect genetic structure and diversity.

2. Material and methods

2.1. Sampling, DNA purification, DNA amplification and species validation

Samples of *T. newnesi* Boulenger, 1902, *T. hansoni* Boulenger, 1902 and *T. bernacchii* Boulenger, 1902 of the family Nototheniidae were collected using land-based sampling in Adelie Land and the Ross Sea and by bottom trawls during various scientific expeditions (Fig. 1, Table 1). For *T. newnesi*, two temporal samples were available from the same site at Adelie Land. Specimens were morphologically identified on site and muscle or fin tissue was preserved in 100% ethanol.

Total genomic DNA was extracted using the Nucleospin Extraction kit (Macherey-Nagel GmBH) following the manufacturer's specifications. Amplification of a set of six microsatellites followed the methods described in Van Houdt et al. (2006). PCR products were visualised on an ABI 3130 Genetic Analyser (Applied Biosystems). Allele size was determined by means of an internal Genescan 500-LIZ size standard and genotypes were obtained using GENEMAPPER v.3.7 (Applied Biosystems). Mitochondrial sequences of the cytochrome *b* gene (*cyt b*) for a subset of individuals were obtained according to the protocol of Janko et al. (2007) using the primers: CytBU15786-Tremato (5′TGAG-GkGGrTTTTCGGTAGATA-3′) and CytB-L16317-Tremato (5′-GATrTAnG-GrTCCTCAaCGGG-3′). Haplotypes were compared to those of Janko et al. (2007). Novel sequences were submitted to genbank JX138967-JX138996. For an overview of the accession number see Table A4–6. Sequences were aligned and analyses were performed in BioEdit v.7.0.9. Morphological identification was validated genetically either using the method described in Van de Putte et al. (2009) or by blasting the *cyt b* sequences. The former method assigns specimens to an *a priori* defined baseline group of eight trematomid species based on samples for which there was a congruent morphological and *cyt b* identification. Analyses were performed in STRUCTURE v.2.3 (Pritchard et al., 2000) using the non-admixture model in combination with the non-correlated allele frequency model. A burn in period of 10^5 steps and 10^6 Markov Chain Monte Carlo simulations was used. A total of 5 runs were carried out to check the repeatability of the results. Assignments scores (q) were plotted and the proportion of correctly assigned individuals ($q > 0.8$) was calculated.

2.2. Microsatellites

2.2.1. Genetic diversity

Genotype and allele frequencies of the microsatellite loci were used to obtain standard estimates of genetic diversity within and between sample sites. Tests for null alleles and other potential technical artefacts, such as stuttering and large allele dropout, were performed

Fig. 1. Geographical distribution of the sampling sites. Sample codes as in Table 1.

using the software package MICRO-CHECKER v.2.2.1 (Van Oosterhout et al., 2004). Genetic variation in each population was measured by calculating the mean numbers of alleles per locus, the observed (H_O) and unbiased expected (H_E) heterozygosities and the F_{IS}. Deviations from Hardy-Weinberg equilibrium (HWE) were assessed with GENETIX v.4.05 (Belkhir et al., 2002). Allelic richness was determined using FSTAT v.2.9.3.2 (Goudet, 2001). Exact tests of linkage disequilibrium between pairs of loci were calculated at each location using GENEPOP v.3.4 (Rousset, 2008). To assess the statistical power of our microsatellite markers compared to the observed levels of differentiation (F_{ST}), we used a modified version of the program POWSIM (Ryman and Palm, 2006) tailored to incorporate a higher numbers of alleles. This program allows to estimate the probability of type I and type II errors for a given set of loci.

2.2.2. Population subdivision

Six complementary methods were used to determine the population structure of *T. newnesi*, *T. hansoni* and *T. bernacchii*. A factorial correspondence analysis (FCA) on multilocus genotypes was performed using GENETIX v.4.05 (Belkhir et al., 2002). Population differentiation was quantified in FSTAT v.2.9.3.2 (Goudet, 2001) using the standard allelic variation F_{ST} estimated as θ (Weir and Cockerham, 1984). To calculate the relationship between geographical distance (coastline distances among sampling sites) and genetic distance (population pairwise F_{ST}) we performed a mantel test in ARLEQUIN v.3.5 (Excoffier and Lischer, 2010). We also plotted a linear regression between geographic distance (LN(distance)) and genetic distance (F_{ST}/(1-F_{ST})) for a visual guidance. Bayesian clustering was performed using STRUCTURE v.2.3.2 to determine the number of populations (K) with the highest posterior probability and to estimate admixture proportions. Simulations were performed using an admixture model with correlated allele frequencies between populations and using sampling location information (MCMC consisted of 20^4 burn in iterations followed by 10^5 sampled iterations). The range of possible tested K was one to ten and five trial runs were carried out for each putative K. K was estimated using the procedure of Evanno et al. (2005). We performed an hierarchical analysis of genetic structure through an Analysis of Molecular Variance (AMOVA) (Excoffier et al., 1992) implemented in ARLEQUIN v3.5 (Excoffier and Lischer, 2010) to test for geographical structure. Using F_{ST} measures of genetic distance, we estimated the level of genetic structuration within populations, between populations within a region, and between regions (which were defined according to the results of STRUCTURE).

In order to detect putative deviations from migration-drift, we applied a Bayesian approach developed by Ciofi et al. (1999) and implemented in the program 2MOD by M.A. Beaumont (http://www.rubic.rdg.ac.uk/~mab/software.html). Two models were evaluated: (1) the gene flow model, which assumes that gene frequencies within populations are caused by a balance between genetic drift and gene flow, and (2) the drift model, in which allele frequencies within populations are evolving purely through drift and differentiation among populations was caused by splitting of ancestral population with no subsequent migration. Both models assume negligible effect of mutations such that alleles are identical by descent, and the gene flow model assumes that the mutation rate is much smaller than the immigration rate (Ciofi et al., 1999). For each population, the program also calculates F (an analogue of F_{ST}), which is the probability of coalescence among randomly chosen chromosomes within the same population. For each species, we analysed two types of dataset: a) treating each sampling site as a separate population and b) pooling the sites according to the STRUCTURE results. The Markov Chain Monte Carlo search was carried out twice on each dataset using 10^5 iterations. The first 10% of the runs were discarded to remove effects of initial starting parameters. In each run, the probability of given model was estimated from the number of times it appeared during the simulation.

Table 1
Description of sampling sites for all species.

Trematomus species	Location	Location code	Sampling year	Expedition name	N_m	N_s
T. newnesi	Cape Hallett	CH	2004	ANZ K066	30	31
	Joinville Island	JI	2007	RV Polarstern ANTXIII/8	49	15
	King George Island	KGI	2007	RV Polarstern ANTXIII/8	15	3
	South Orkneys	SO	2006	RV James Clark Ross AFI 6/16	43	6
	Adelie Land I	TA1	2003	land based / ICOTA	89	0
	Adelie Land II	TA2	2005	land based / ICOTA	57	20
T. hansoni	Cape Hallett	CH	2004	ANZ K066	33	27
	Cape Roberts	CR	2002	ANZ K066	9	7
	Joinville Island	JI	2007	RV Polarstern ANTXIII/8	15	22
	South Georgia	SG	2007	S. Georgia Groundfish Survey SG07	21	10
	South Orkneys	SO	2006	RV James Clark Ross AFI 6/16	5	4
	Adelie Land	TA	2003	land based / ICOTA	19	3
T. bernacchii	Cape Armitage	CA	2002	ANZ K066	42	11
	Cape Roberts	CR	2002	ANZ K066	28	10
	Casey	CS	2008	Australian Antarctic Division	43	17
	Deception Island	DI	2006	RV James Clark Ross AFI 6/16	7	2
	Elephant Island	EI	2007	RV Polarstern ANTXIII/8	5	5
	Joinville Island	JI	2007	RV Polarstern ANTXIII/8	5	5
	South Orkneys	SO	2006	RV James Clark Ross AFI 6/16	5	5
	Adelie Land	TA	2003	land based / ICOTA	12	11
	Rothera	RO	2006	BAS	12	8

N_m: sampling size used for microsatellite analysis, N_s: sampling size used for *cytochrome b* sequence analysis.

2.2.3. Inferences of past population size changes

To detect deviations from mutation-drift equilibrium indicating recent population oscillations from microsatellite markers, the mode-shift indicator test was employed to detect genetic bottlenecks using BOTTLENECK v.1.2.02 (Cornuet and Luikart, 1997). The test assumes that a population under mutation-drift equilibrium is expected to have a larger proportion of alleles with low frequencies than an expanding population. Due to limited sample size at some sampling sites, the test was performed for the genetically homogenous population clusters as indicated by STRUCTURE.

2.3. Mitochondrial DNA analysis

2.3.1. Genetic diversity

The optimal model for sequence evolution for each dataset of *cyt b* was assessed through jModeltest (Posada, 2008) and used to measure the Φ_{ST} over all populations in ARLEQUIN v.3.5, which is analogous to Wright's F_{ST}, but takes into account the divergence between haplotypes. We tested whether the geographic partitioning observed from nuclear markers also explains a significant portion of mtDNA variability. To do so, we applied AMOVA in ARLEQUIN to estimate the level of genetic structure within populations, between populations within a region, and between regions, defined according to STRUCTURE (see below). Appropriate values of F statistic components were calculated from Φ values (Excoffier et al., 1992).

The relative likelihoods of a model of immigration-drift equilibrium versus drift was determined using 2MOD (see above). We recoded the presence/absence of haplotypes into a haplotype per population matrix and analysed two types of datasets for each species; a) treating each sampling site as a separate population and b) pooling the sites according to design by the FCA/STRUCTURE analysis of nuclear markers. The Markov chain Monte Carlo search was carried out as described above.

2.3.2. Inferences of past population size changes

Two neutrality tests were employed to detect traces of historical demographic changes. These tests, originally developed to test the neutral hypothesis under an infinite-site model with constant population size scenario, are known to be sensitive to past population size changes causing deviation from the mutation-drift equilibrium. The Tajima's D test of neutrality (Tajima, 1989) evaluates the significance of two estimators of population parameter θ, one estimated from the number of segregating sites, the other from π. D is expected to be negative if the population has experienced an expansion. Fu's Fs test (Fu, 1997), evaluates the probability of sampling the same or lower amount of alleles as in a sample from neutral population assuming the same number of pairwise differences as in the studied sample. It is expected to produce negative values in expanded populations and is considered as one of the strongest methods for detecting traces of simulated logistic as well as stepwise expansion models (Ramos-Onsins and Rozas, 2002). Both tests were performed in DnaSP v.5.0 (Librado and Rozas, 2009) and their significance was estimated via 1000 implemented coalescent simulations. Given value was considered as significant if the probability of its occurrence in neutral population of stable size was lower than 0.05.

Finally, we used a Markov Chain Monte Carlo (MCMC) approach implemented in FLUCTUATE v.1.4 (Kuhner et al., 1998) to estimate the Maximum Likelihood of the exponential growth parameter g ($\theta_t = \theta_{initial}\ ^{-gt}$) for our datasets. The significance of ML estimate can be judged from likelihood surface provided by Fluctuate (Kuhner, 2003) The log likelihood output table shows an approximate 95% confidence interval consisting of all values of and g that lay within the 2 units of the maximum likelihood estimate. If $g = 0$ occurs within that interval of values of the best fitting , than is the population plausibly not expanding. Furthermore, the approximate of standard deviation gives an idea of expected error around the estimate. We conservatively considered that a given dataset bears a signal of significant population growth if the ML estimate of g was greater than three standard deviations (Lessa et al., 2003).

3. Results

3.1. Microsatellites

3.1.1. Suitability of the data to evaluate population structure

The power to detect population differentiation given the observed global F_{ST}s (see below) and the sampling scheme for each species was assessed with the software package POWSIM. Estimates of α-error with the current sampling design were low (<5%) and the power approached unity in all species suggesting that our sampling scheme was adequate to detect deviations from panmixia given the observed variability of markers.

Three loci, Trne035F22A, Trne055F55A and Trne066F58A showed significant heterozygote deficiency after Bonferroni correction in some species-locality combinations (see below and Tables A1–A3) but no

Table 2
Estimates of genetic diversity based on microsatellite and mtDNA genotypes of *T. newnesi*, *T. hansoni* and *T. bernacchii*.

Species/ population	microsatellites				mtDNA		
	$H_{E\ n.b.}$	H_O	F_{IS}	R_A	alleles	Hd	π
T. newnesi					Tamura and Nei + Γ (Γ = 0.011)[a]		
CH	0.831	0.856	−0.030	9.430	10	0.809	0.0617
JI	0.868	0.847	0.025	10.930	10	0.895	0.0768
KGI	0.841	0.724	**0.143**	9.154	2	1	0.0032
SO	0.846	0.783	**0.076**	10.437	3	0.600	0.0930
TA1	0.849	0.823	0.031	9.768	11	0.926	0.0474
TA2	0.856	0.860	−0.004	9.780			
T. hansoni					Kimura 2 parameter[a]		
CH	0.716	0.703	0.018	4.55	7	0.655	0.0046
CR	0.766	0.722	0.060	4.68	2	0.571	0.0029
JI	0.740	0.789	−0.068	4.63	9	0.797	0.0038
SG	0.660	0.531	0.199	4.18	4	0.733	0.0034
SO	0.730	0.567	0.244	5.00	3	0.833	0.0038
TA	0.696	0.743	−0.070	4.30	2	0.667	0.0017
T. bernacchii					Tajima and Nei + Γ (Γ = 0.1278)[a]		
CA	0.725	0.725	0	4.63	2	0.182	0.0004
CR	0.746	0.774	−0.039	4.77	4	0.533	0.0021
CS	0.821	0.764	**0.071**	5.03	5	0.574	0.0018
DI	0.705	0.762	−0.088	4.43	1	0	0
EI	0.611	0.567	0.081	3.52	1	0	0
JI	0.726	0.667	0.091	4.77	1	0	0
RO	0.792	0.792	0.001	4.98	3	0.464	0.0026
SO	0.631	0.708	−0.146	4.00	1	0	0
TA	0.721	0.706	0.023	4.60	2	0.182	0.0004

$H_{E\ n.b.}$: expected heterozygosity, H_O: observed heterozygosity, F_{IS}: Fixation index according to Weir and Cockerham (1984), R_A: allelic richness, alleles: number of alleles, Hd: haplotype diversity, π: mean pairwise divergence for each population,
[a] The best-fit model of sequence evolution indicated for each species by jModeltest for the mtDNA dataset. See Table 1 for sample codes. Bold values: significant F_{IS} values after Bonferroni correction.

marker pointed to a consistent pattern of heterozygote deficiency in more than 2 localities. MICROCHECKER analysis indicated that null alleles may be present at these loci. Calculations on the original data were replicated using corrected allele frequencies according to Brookfield (1996). Replicates using the corrected allele frequencies did not change the results and were not retained for further analyses. Pairwise comparisons between loci revealed no significant linkage disequilibrium after Bonferroni corrections.

In each nuclear dataset, the 2MOD analysis suggested that the model assuming migration-drift equilibrium is more likely (probabilities > 0.99) than the non-equilibrium model. Hence, measures of genetic differentiation do reflect ongoing gene flow among populations. In the *T. bernacchii* dataset, modal F values ranged between 0.0047 (JI) to 0.0880 (DI) with the exception of EI sampling site (F = 0.1950). In the *T. hansoni* dataset, the F values were similar, ranging between 0.0014 (CR) and 0.0727 (SO) except for the SG population (F = 0.1325). In the *T. newnesi* total dataset, the F values ranged between 0.0063 (JI) to 0.0350 (KGI).

3.1.2. Genetic diversity

Mean allelic richness corrected for sample size was highest for *T. newnesi* (9.15–10.93) while *T. hansoni* (4.18–5.00) and *T. bernacchii* (3.52–5.03) were characterised by similar levels of allelic richness. *T. newnesi* displayed relatively uniform range in mean expected heterozygosity among sampling locations (0.81–0.86) which was generally higher than the wider ranges of *T. hansoni* (0.64–0.72) and *T. bernacchii* (0.55 – 0.81). Significant departures from Hardy–Weinberg equilibrium after Bonferroni correction were observed in two populations of *T. newnesi* (KGI and SO), one population of *T. bernacchii* (CS) and none of the *T. hansoni* populations (Table 2, Tables A1–A3).

3.1.3. Population structure

Testing of the stepwise clustering procedure performed in STRUCTURE for *T. newnesi* resulted in the separation of two hypothetical clusters (highest Δk for k = 2). There was a distinct separation between the cluster of samples from the High Antarctic composed of the Ross Sea and Adelie Land area (CH, TA1, TA2,) and the cluster of samples from the Peninsula region (KGI, JI, SO). *T. hansoni* and *T. bernacchii* each showed three clusters (highest Δk for k = 3). Specifically, *T. hansoni* showed a separation between the High Antarctic (TA, CH, CR) and the peninsula region (JI, SO) similar to *T. newnesi* but with an additional cluster for the more northern sample at South Georgia. For *T. bernacchii*

Table 3
Summary of the assignment analysis following STRUCTURE based on microsatellite genotypes of *T. newnesi*, *T. hansoni* and *T. bernacchii*.

	Population[a]	Cluster 1	Cluster 2	Cluster 3
T. newnesi				
	TA1	0.249	**0.751**	
	TA2	0.207	**0.793**	
	CH	0.306	**0.694**	
	KGI	**0.830**	0.170	
	JI	**0.860**	0.140	
	SO	**0.868**	0.132	
T. hansoni				
	TA	0.139	**0.824**	0.037
	CH	0.223	**0.735**	0.041
	CR	0.349	**0.562**	0.089
	JI	**0.915**	0.053	0.032
	SO	**0.917**	0.058	0.025
	SG	0.225	0.029	**0.746**
T. bernacchii				
	CS	**0.939**	0.041	0.021
	TA	0.245	0.194	**0.561**
	CA	0.187	0.198	**0.614**
	CR	0.145	0.261	**0.595**
	RO	0.275	**0.644**	0.081
	DI	0.031	**0.857**	0.111
	JI	0.067	**0.702**	0.231
	EI	0.026	**0.901**	0.073
	SO	0.050	**0.719**	0.231

[a] Sample codes as in Table 1, bold values: statistical significant values after Bonferroni correction.

there was a distinct cluster for Casey (CS) as well as a further separation between a high Antarctic cluster consisting of the Ross Sea and Adelie Land samples (CA, CR, TA) and the cluster of the Peninsula region samples (RO, EI DI, JI,SO) (Table 3, Fig. 2). For all species the FCA clustering resulted in a similar grouping of populations with clear separation of Peninsular and the High Antarctic clusters (Fig. 3). Note that for *T. newnesi* both temporal samples from the same locality (TA1 and TA2) appeared close to each other.

The population structure according to the STRUCTURE and FCA analysis was tested in AMOVA. For the pelagic *T. newnesi* the analysis revealed that the among-group component (F_{ct}) explained only 0.24% of total genetic variation and this result was not significant. For both benthic species the AMOVA found significant amount of among-group partitioning of genetic variance, explaining 8.58% in *T. hansoni* and 3.51% for *T. bernacchii*.

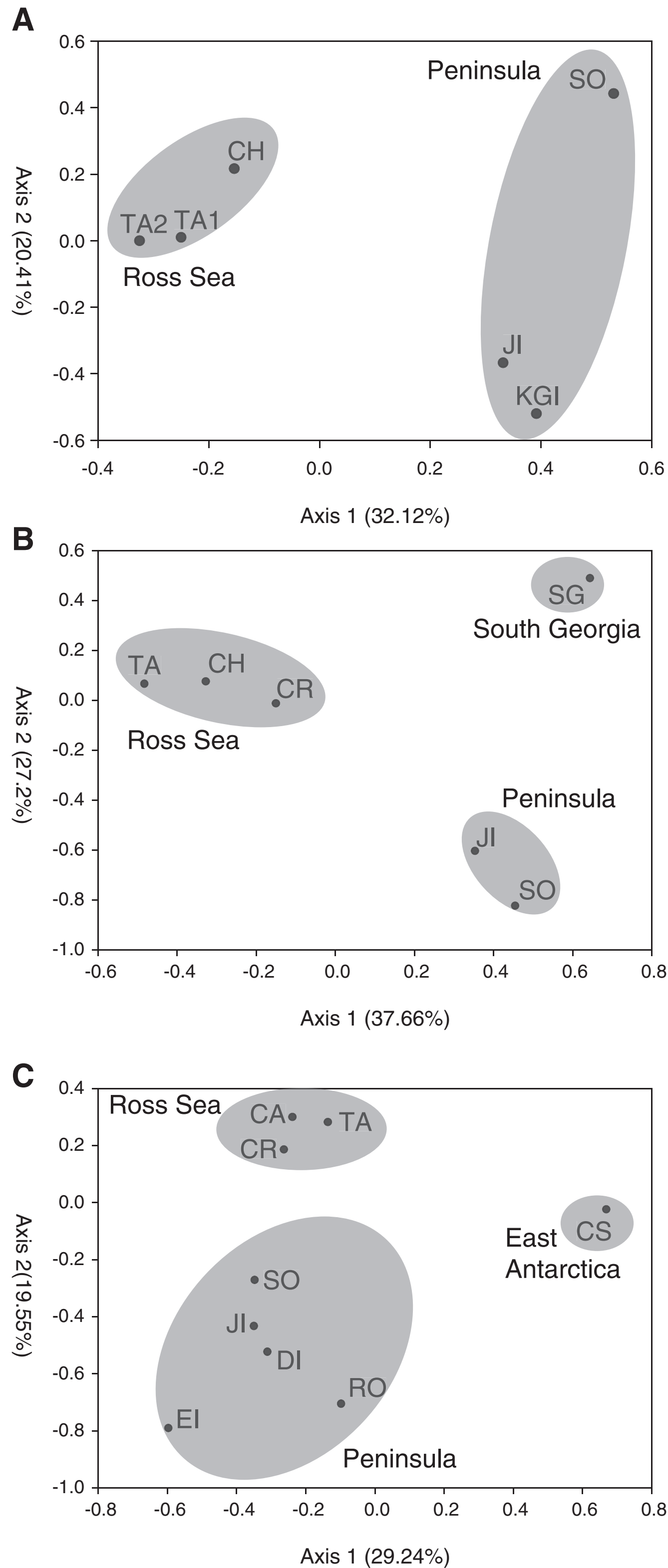


Fig. 2. Plot of the clustering analysis conducted in STRUCTURE (Pritchard et al., 2000) based on the microsatellite data of *Trematomus newnesi* (A) (K = 2), *T. hansoni* (B) (K = 3) and *T. bernacchii* (C) (K = 3). Each individual is represented by a vertical bar indicating its estimated proportion of membership to each cluster. Sample codes as in Table 1.

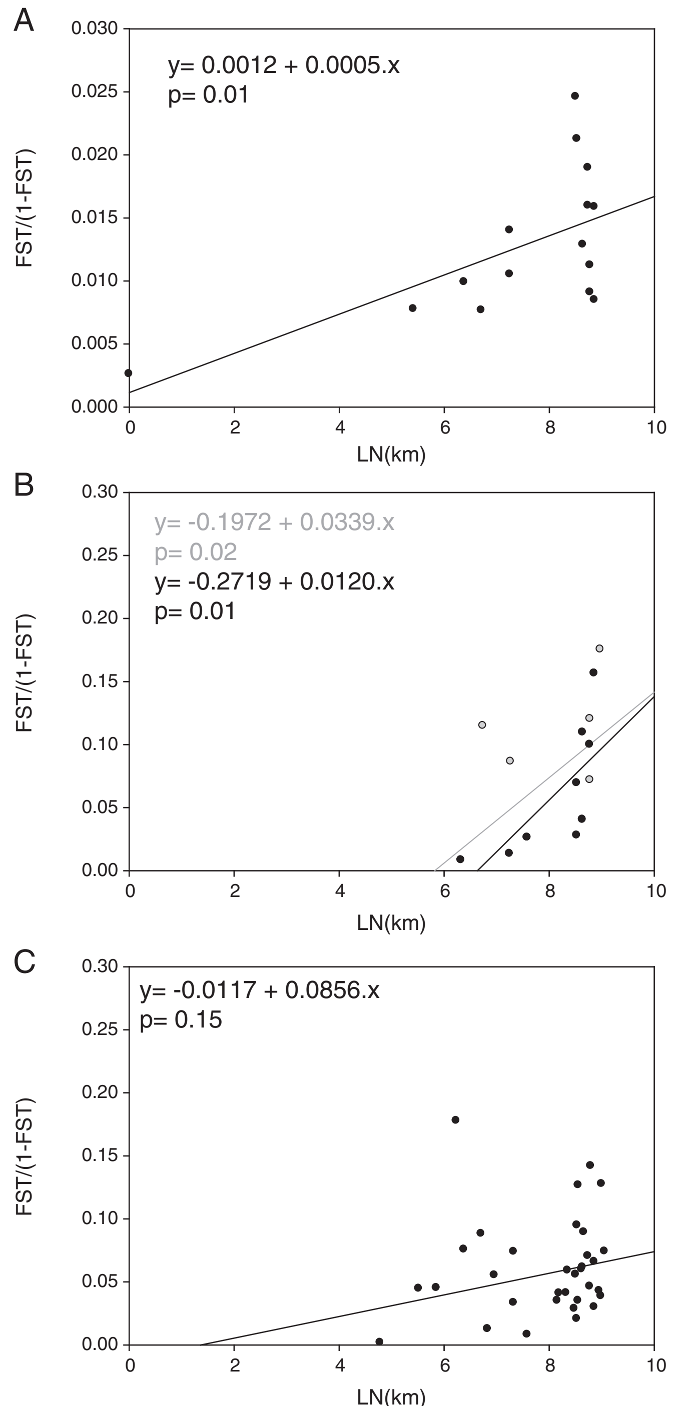


Fig. 3. Factorial correspondence analysis plot based on microsatellite data of *Trematomus newnesi* (A), *T. hansoni* (B), *T. bernacchii* (C). Sample codes as in Table 1.

Table 4
Population pairwise F_{ST} (Φ_{ST}) values of *T. newnesi* samples. The upper triangular matrix lists the values for microsatellite genotypes, and lower triangular lists the values for mtDNA genotypes.

T. newnesi[a]	CH	JI	KGI	SO	TA1	TA2
CH		**0.0208**	**0.024**	**0.0127**	**0.0104**	**0.0138**
JI	0.0854		0.0077	0.0098	**0.0090**	**0.0111**
KGI	0.0166	0.1926		0.0076	**0.0157**	**0.0186**
SO	−0.0453	0.1597	0.1535		**0.0084**	**0.0156**
TA1	−0.0164	−0.0032	−0.0339	−0.1425		0.0026

[a] Sample codes as in Table 1, bold values: statistical significant values after Bonferroni correction.

Table 5
Pairwise F_{ST} (Φ_{ST}) values of *T. hansoni* samples. The upper triangular matrix lists the values for microsatellite genotypes, and lower triangular lists the values for mtDNA genotypes.

T. hansoni[a]	CH	CR	JI	SG	SO	TA
CH		0.0081	**0.0647**	**0.1073**	**0.0986**	0.013
CR	**0.3032**		0.027	**0.0668**	0.0387	0.0254
JI	0.0485	**0.2881**		**0.0794**	−0.0077	**0.0907**
SG	0.0213	**0.4759**	**0.0805**		**0.1029**	**0.1492**
SO	−0.0064	**0.3913**	−0.0210	0.0070		**0.1352**
TA	−0.0094	**0.5478**	0.0396	−0.0632	−0.0367	

[a] Sample codes as in Table 1, bold values: statistical significant values after Bonferroni correction.

The pattern of the pairwise F_{ST} values of *T. newnesi* resembled the STRUCTURE analysis (Table 4): no significant differentiation was observed within the Peninsula region as well as between the two temporal samples from TA. Overall F_{ST} was 0.0104 (0.0071–0.0147). Global F_{ST} for *T. hansoni* was 0.0717 (0.0320–0.1240) or 0.0459 (0.0105–0.1046) when excluding the SG sample. All pairwise F_{ST} values that include SG were significant after Bonferonni correction. There were no significant pairwise differentiations within the High Antarctic or within the Peninsula clusters (Table 5). The Global F_{ST} of *T. bernacchii* was similar to that of *T. hansoni* excluding the SG samples: 0.0383 (0.01577–0.07169). No significant F_{ST} values were observed within both High Antarctic and Peninsula clusters (Table 6).

In all species the pairwise F_{ST} values correlated with the log transformed distance between sampling locations, suggesting a pattern indicative of isolation-by-distance (IBD; Fig. 4, Table 7). Although analysis of all *T. hansoni* samples revealed a significant IBD pattern we also excluded the most divergent SG population, in order to account for the possible effect of long-term isolation among populations (de Campos Telles et al., 2005). This lead to a strengthening of the signal ($r = 0.79$, $p < 0.01$). A Global Mantel test including all *T. bernacchii* samples revealed only a marginally significant pattern of IBD ($r = 0.27$, $p = 0.054$), which, however, became highly significant after excluding an outlying value related to the EI sample (Fig. 4, Table 7).

3.1.4. Demography

Bottleneck analysis found that both population clusters of *T. newnesi* had a normal L-shaped distribution of allele frequencies as expected under mutation-drift equilibrium and constant population size. For *T. hansoni* this analysis indicated recent growth in two population clusters including SG and those located around the Peninsula, while no deviations from equilibrium were detected in the High Antarctic cluster. We also found evidence of demographic fluctuations for *T. bernacchii* in the Peninsula region, while no deviations from mutation-drift equilibrium were detected in either High Antarctic clusters of the Ross Sea-Adelie Land and Casey (Fig. 5).

3.2. Mitochondrial DNA

jModeltest selected as best models for sequence evolution in *T. newnesi*, *T. hansoni* and *T. bernacchii*, respectively, the following mutational models: Tamura&Nei + Γ ($\Gamma = 0.011$), Kimura-2-parameter and Tajima&Nei + Γ ($\Gamma = 0.1278$). Treating each population separately, the mtDNA 2MOD analysis favoured the gene-flow-drift scenario over the pure-drift scenario; the probability of the model 1 equalled 0.98 in *T. newnesi*, 0.73 in *T. hansoni* and 0.79 in *T. bernacchii*. The probability of the model 1 equalled unity when populations were clustered according to the design suggested by the STRUCTURE. The haplotype diversity was highest in *T. newnesi* varying between 0.60 and 0.93, while in *T. hansoni* and *T. bernacchii* it ranged between 0.57–0.83 and 0–0.57, respectively. Similarly, the nucleotide diversities were highest among *T. newnesi* samples ranging between 0.0032 and 0.0930, while in *T. hansoni* and *T. bernacchii* we observed lower values between 0.0017–0.0046 and 0–0.0026, respectively (Table 2). Global (Φ_{ST}) values were lowest in *T. newnesi* (0.03), intermediate in *T. hansoni* (0.13) and highest in *T. bernacchii* (0.51).

The geographical partitioning of *T. newnesi* mtDNA variability was weak, since the F_{ct} component of AMOVA accounted for only 8% of the total variability and was not significant. Similarly, in *T. hansoni*, we found 0% of explained variance, indicating no detectable effect of population structure on mtDNA among the regions. In contrast, the F_{ct} component accounted for 44% of total variance in *T. bernacchii*, which was highly significant.

The global Mantel test for the *T. newnesi* mitochondrial dataset suggested a pattern corresponding to IBD which was not clearly significant ($p = 0.07$) (Table 7). Similarly, a global Mantel test including all *T. bernacchii* samples revealed only a marginally significant pattern of IBD ($r = 0.27$, $p = 0.054$). After excluding an outlying value related to the EI sample, we observed a highly significant pattern of IBD in this species (Table 7). On the contrary, the global Mantel test for *T. hansoni* did not reveal any IBD pattern, even when excluding the SG sample.

All datasets indicated the deviations from mutation-drift equilibrium suggestive of population oscillations, albeit with different intensity. We found negative but non-significant values of Tajima's D (D=

Table 6
Pairwise F_{ST} (Φ_{ST}) values of *T. bernacchii* samples. The upper triangular matrix lists the values for microsatellite genotypes, and lower triangular lists the values for mtDNA genotypes.

T. bernacchii[a]	CA	CR	CS	DI	EI	JI	SO	TA	RO
CA		0.0016	**0.0393**	0.0527	**0.1123**	0.0338	0.0819	−0.0016	**0.0556**
CR	**0.0523**		**0.0338**	0.0278	**0.0866**	0.0201	0.0579	0.0079	0.0394
CS	0.0064	−0.0360		**0.041**	**0.1131**	0.0372	0.0689	**0.0322**	**0.029**
DI	−0.3253	−0.2690	−0.2714		0.0431	−0.0085	0.0809	0.0657	−0.0003
EI	**0.8840**	**0.5259**	**0.5392**	1.0000		0.0426	0.1508	0.1242	0.0522
JI	**0.8840**	**0.5259**	**0.5392**	**1.0000**	0.0000		0.0702	0.0442	0.0123
SO	**0.8892**	**0.5456**	**0.5429**	**1.0000**	0.0000	0.0000		0.0618	0.0687
TA	**0.0002**	**0.0525**	0.0283	−0.3256	**0.8840**	**0.8840**	**0.8833**		**0.0564**
RO	**0.5946**	**0.3310**	**0.3846**	0.3773	−0.0833	−0.0833	−0.0414	**0.5946**	

[a] Sample codes as in Table 1, bold values: statistical significant values after Bonferroni correction.

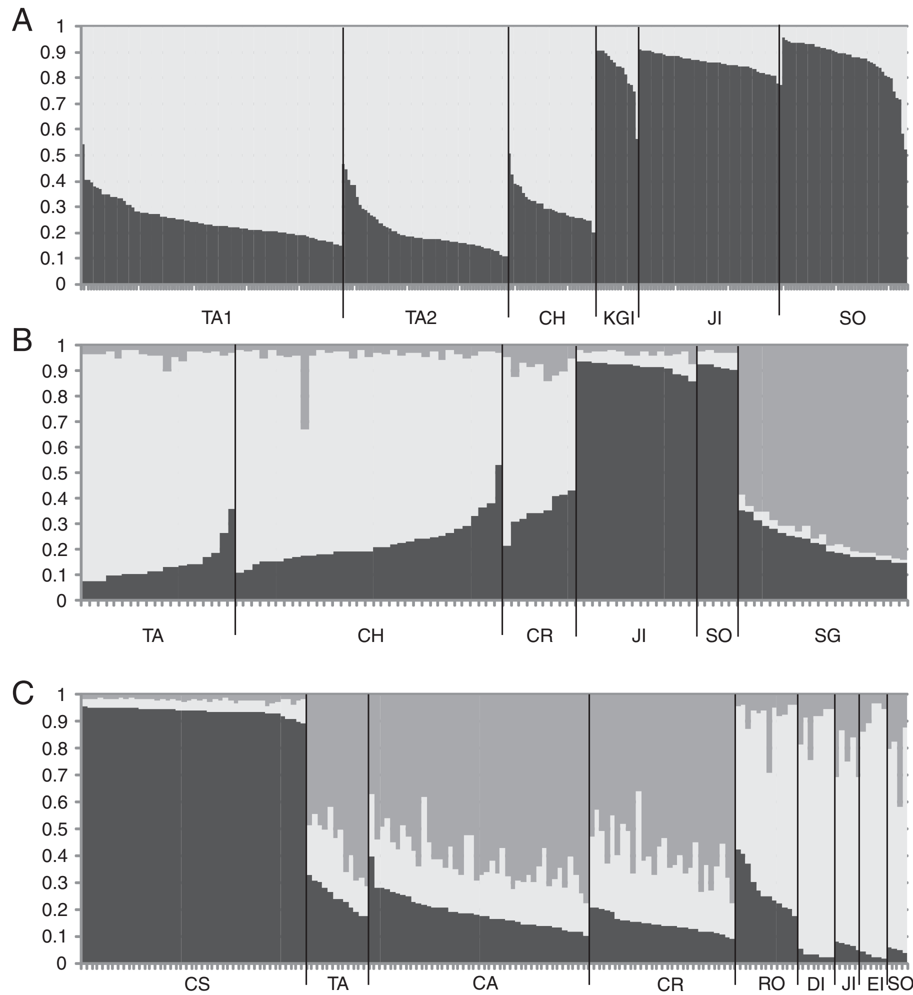


Fig. 4. Genetic isolation by distance inferred from F_{ST} genetic distances versus geographical distance for *Trematomus newnesi* (A), *T. hansoni* (B), *T. bernacchii* (C).

−1.06) in *T. newnesi* and the g parameter of the expansion model was 369 (3 SD = 89) and the lnL values of the MLE and zero growth were similar. However the Fu's F_s value was significantly negative (F_s = −8.96). On the other hand, *T. hansoni* and *T. bernacchii* mtDNA datasets significantly deviated from null expectations according to both Tajima's D (D = −1.76 and −1.49, respectively) and Fu's F_s (F_s = −7.36 and −4.06, respectively) tests and we found g values higher than three s.e. (1263; 3 SD = 189 respectively 3969; 3 SD = 378) indicating significant population growth in both species.

Table 7
Isolation by distance: Mantel test results genetic distance (F_{ST}) with geographic distance (ln(km)) for microsatellite and cytochrome *b* genotypes of *T. newnesi*, *T. hansoni* and *T. bernacchii*.

Trematomus species	Microsatellite		*cytb*	
	R	p	r	P
T. newnesi	**0.63**	**0.02**	0.6814	0.07
T. hansoni	**0.60**	**0.03**	−0.0078	0.49
T. hansoni (excl. SG[a])	**0.79**	**0.001**	−0.1046	0.73
T. bernacchii	*0.27*	*0.054*	**0.3109**	**0.03**
T. bernacchii (excl. EI[a])	**0.52**	**0.01**	**0.5165**	**0.01**

r: correlation coefficient, p: probability of correlation coefficient.
[a] For sample codes see Table 1, bold values: statistical significant values after Bonferroni correction.

4. Discussion

4.1. Quality of the data

Population structure inferred from nuclear as well as mtDNA markers was lower in *T. newnesi* than in either benthic species, which is consistent with the hypothesis that the genetic structure of marine fish is affected by ecological specialisation. However, before proceeding with a discussion on the biological relevance of our findings, we have to address several issues potentially interfering with the interpretation of the data. Significant deviations from HWE due to homozygote excess were observed at three out of six microsatellite loci in two species. These may be attributed to a number of factors, such as population substructuring or admixture, inbreeding, selection at or near a microsatellite locus, small sample size or null alleles, which are a common artefact of microsatellites (O'Reilly et al., 2004). However, none of the loci displayed homozygote excess in a large number of populations, which would be expected under the locus-specific problems and the overall results did not change when implementing corrected distances. Further, the power analysis suggested that our sampling scheme was adequate to detect deviations from panmixia under the observed range of F_{ST}'s.

Although differences in mitochondrial sequences between benthic and pelagic species observed from mitochondrial sequences are generally consistent with the microsatellites, global as well as pairwise Φ_{ST} values were usually higher than corresponding estimates of F_{ST} from nuclear markers. This difference is most notable in the case of *T. bernacchii*, where the overall Φ_{ST} and F_{ST} differed by an order of magnitude.

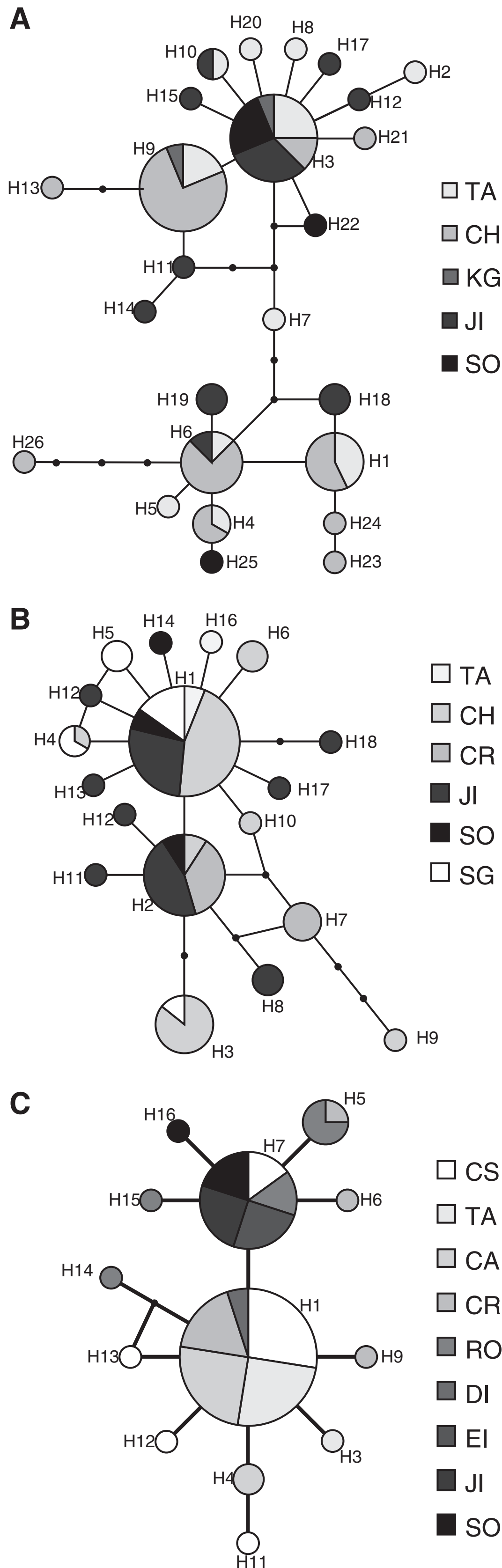


Fig. 5. Haplotype networks of data of *Trematomus newnesi* (A), *T. hansoni* (B) and *T. bernacchii* (C). Sample codes as in Table 1.

Contrasts in genetic differentiation between mitochondrial and nuclear markers are commonly observed in other organisms (Rogers, 2007; Larmuseau et al., 2010) and may be attributed to a number of non-neutral mechanisms, such as sex-specific dispersal or selection (Bazin et al., 2006). Buonaccorsi et al. (2001) demonstrated by simulation that substantial differences in F_{ST} estimates from nuclear and mtDNA markers might arise from different modes of inheritance and genomic sampling variances. Observed Φ_{ST} value of 0.43 in *T. bernacchii* is well within the 95% C.I. predicted by Buonaccorsi et al. (2001) simulations for a nuclear F_{ST} value of 0.038. Therefore, sex-specific dispersal or locus-specific selection do not have to be invoked to account for observed differences between mtDNA and nuclear markers. Indeed, we have no evidence for sex-specific dispersal in Trematominae. Janko et al. (2007) found no evidence of locus-specific selection when comparing *cytb* and nuclear DNA sequences in *T. bernacchii* and *T. newnesi*.

Finally, genetic measures of population differentiation are sensitive to demographic contraction/expansion events, and genetic drift and gene flow may take a long time to equilibrate (Hellberg, 2009). Traces of historical population fluctuations are evident in both benthic species and to a lesser extent also in the *T. newnesi* dataset given the negative mitochondrial Fu's F_s. Although such fluctuations might have obscured real isolation of contemporary populations, the 2MOD analysis unambiguously favours the model implying a balance between migration and drift in local demes, suggesting that analyses of genetic differentiation reflect on-going dispersal rather than past expansion/contraction/fragmentation events.

4.2. Spatio-temporal partitioning of genetic variability

There is increasing evidence that Southern Ocean fish display not only geographical variability, but also considerable temporal variability in the genetic composition of recruits (Zane et al., 2006; Papetti et al., 2007, 2009; Patarnello et al., 2003). This may result from sweepstakes effects. These effects are caused by stochastic larval mortality when only a small proportion of the available gene pool successfully contributes to the replenishment of the population (Hedgecock, 1994). However, we found no evidence of a sweepstakes effect in our study. This is shown by samples of *T. newnesi* from Adelie Land taken at different times at the same locality. These samples displayed the lowest pairwise F_{ST} among all samples. In short, to the best of our knowledge, the interspecific comparison of the species should not be largely influenced by a time-dependent effect on sampling.

According to our analyses of nuclear markers, patterns of geographical structure were similar among all three species. In *T. newnesi*, the STRUCTURE analysis identified two separated regions including the Peninsular sites and the sites in the High Antarctic (which is also generally consistent with the FCA). Both benthic species were separated into three clusters, two of which matched the above-mentioned population groupings, while the third cluster corresponded to a site that was marginal in our sampling scheme, i.e. the eastern-most site near the Casey station in *T. bernacchii* and the northernmost location in the South Georgia in the case of *T. hansoni*. In all species, significant pairwise F_{ST} values exclusively concerned comparisons between samples from distinct regions. Despite the large distance among sampling sites, 2MOD analyses revealed rather low modal F values in comparison with other organisms. The two populations with relatively high F values (EI in *T. bernacchii* and SG in *T. hansoni*) were also identified by pairwise F_{ST} estimates as the most aberrant ones. While the low sample size from EI may explain the former observation, the outlying character of the SG population may reflect effective isolation by the Polar Front (Shaw et al., 2004).

The geographical structure identified in this study matches traditional biogeographic separation of the Southern Ocean (Barnes and De Grave, 2000) implying the existence of barriers to dispersal among distinct regions. Alternatively, observed patterns may result from the process of IBD combined with the absence of samples from

the zones between Peninsular and High Antarctic sites. Significant results of the Mantel tests seem consistent with this possibility. Unfortunately, this issue may not be satisfactorily settled given the sampling limitations.

4.3. Ecology-driven differences in gene flow

Contemporary genetic studies of high-Antarctic notothenioids often report the lack of significant differentiation among sites separated by thousands of kilometres (see Matschiner et al., 2009; Patarnello et al., 2011; Papetti et al., 2012). Genetic homogeneity is supposed to be maintained across large distances passive by larval dispersal through currents. Indeed Matschiner et al. (2009) showed that such passive dispersal is sufficient to homogenise the populations of benthic fish over a transect spanning from the South Shetlands to South Georgia. We found significant interregional differences in all three species over a transect length rarely considered in this paper was rarely achieved in previous studies. For example, the analysis of Matschiner et al. (2009) and Papetti et al. (2012) were restricted to the region more or less corresponding to our "Peninsular population cluster". Here our samples generally did not significantly deviate from panmixia. The significant differentiation of the South Georgia population of *T. hansoni* contrasts with the non-significant results from *Gobionotothen gibberifrons* (Matschiner et al., 2009) but it is unclear whether such a difference reflects species-specific characteristics or simply insufficient sampling coverage (8 vs. 21 samples in this study). In any case, the present discovery of significant interregional differences suggests that processes determining geographical structure may operate on larger scales than usually considered in genetic studies in the Southern Ocean.

Our study revealed stronger population structuring in *T. hansoni* than in *T. newnesi*, despite the suspected much longer larval period in the former species. When excluding the South Georgia samples of *T. hansoni* from our analysis both benthic species displayed largely similar patterns. Furthermore recent barcoding studies indicate that *Trematomus vicarius*, which is endemic to South Georgia is genetically similar to *T. bernachii* and may constitute an isolated population of the former (Dettai et al., 2011; Lautredou et al., 2010). Thus our data do not entirely conform to the hypothesis that population connectivity of Southern Ocean fish is mainly driven by passive larval dispersal. The weaker level of spatial structuring found for the pelagic *T. newnesi* compared to both benthic species is apparent from lower global as well as pairwise F_{ST} and Φ_{ST} values. The interregional partitioning of genetic variability explained only a fraction (0.25%) of the total nuclear variability and was not significant for *T. newnesi*, while it explained more than 3% of the total variance in both benthic species. This finding suggests that factors other than length of larval period, such as ecology of adult stages or eggs, contribute to interspecific differences in population connectivity among notothenioid fish. However currently the knowledge of life history traits in Southern Ocean fish species is limited and would require further investigation in order draw more in depth conclusions. Differences between pelagic and benthic species found in this study are consistent with a microsatellite-based study of the mesopelagic lanternfish *Electrona antarctica* (Van de Putte et al., 2012), which covered a comparable portion of the Southern Ocean and showed no differentiation. Worldwide pelagic fishes show considerably lower levels of differentiation than benthic and coastal taxa (Ruzzante et al., 2006). Hence, our data are consistent with the hypothesis that the genetic structure of marine fish is affected by ecological specialisation of the species.

It remains unclear, however, whether the different dispersal potential of pelagic versus benthic notothenioids may translate into different adaptive potential or rates of speciation. In many adaptive radiations including notothenioids, allopatric or peripatric speciation might have been promoted by the interplay between the effects of adaptation to local conditions and barriers disrupting the genetic connectivity among populations (Rico and Turner, 2002). Population genetics tools allow the evaluation of population connectivity through estimates of the absolute number of migrants ($Nm \sim (1\text{-}F_{ST})/4F_{ST}$ (Lowe and Allendorf, 2010). Even though such estimates are based on simplifying assumptions and should be considered carefully (for example, the F_{ST}–based counts assume an infinite-island model with equal migration rates and population sizes among demes, while coalescent samplers are sensitive to the effects of ghost populations (Slatkin, 2005)), it is remarkable that our data indicate large levels of gene flow. Except for the mtDNA dataset in *T. bernacchii*, all estimates of geneflow (Nm) were greater than 1, which is usually assumed sufficient to ensure sharing of the same alleles among populations over long periods of evolutionary time. In fact, all Nm estimates were greater than 0.1, which is supposed to be enough for the spread of most adaptive alleles (Rieseberg and Burke, 2001). Hence, inferred levels of gene flow between contemporary populations seem sufficient to maintain genetic cohesiveness within all species.

In the past levels of gene flow may have changed dramatically during the periods, when available habitats have been reduced either by the expansion of the grounded ice sheets or mass wasting and turbidity currents, or through the shutdown of primary productivity under multiannual sea ice (Thatje et al., 2008). Footprints of population fluctuations putatively related to glacial periods are commonly observed in genetic studies of organisms from the Southern Ocean and there is evidence that some organisms not only experienced bottlenecks during the LGM but have been restricted into more or less isolated refugia (rev. in Rogers, 2007, 2012). Janko et al. (2007) showed that *T. bernacchii* mtDNA variability is consistent with the hypothesis of temporal fragmentation into inhabitable refugia whose isolation might have been further strengthened by a decrease of oceanic current activities during glacial periods (Kojima et al., 1997). Bottleneck analyses further indicate that population oscillations may occur asynchronously in distinct regions, since we found evidence of population expansion in northerly sites of both benthic species, while populations in the High Antarctic didn't deviate from mutation-drift equilibrium. Antarctic species therefore probably pass through periods of transient allopatry (Patarnello et al., 2011) similar to mid and high latitude taxa (Hellberg, 1998; Maggs et al., 2008).

Given that pelagic organisms probably were not as much affected by glaciations than benthic organisms (Janko et al., 2007), it is tempting to speculate that periods of transient allopatry might have promoted allopatric speciation especially in benthic feeders (Rogers, 2007, 2012). However, both mitochondrial phylogroups of *T. bernacchii* that putatively originated in isolated refugia underwent postglacial expansion with secondary admixture (Janko et al., 2007); note that both major *cytb* lineages are shared between the regions, albeit in different frequencies. A similar situation has been observed in Adelie penguins (Ritchie et al., 2004). In a multiocus analyses of the Trematominae, Janko et al. (2011) found no evidence for change of speciation or extinction rates in relation to Pleistocene glaciations. Altogether it seems that although the Quaternary climatic fluctuations had a dramatic effect on population size and structure especially in benthic fish (Zane et al., 2006; Janko et al., 2007; Rogers, 2007), they did not provoke mass extinctions or vicariant speciation and postglacial dispersal among temporarily fragmented populations was strong enough to prevent the achievement of reciprocal monophyly.

5. Conclusions

The present comparative analysis provides strong support for the hypothesis that the ecology of Antarctic marine species decisively influences their population structure, leaving benthic species more prone to local disturbances and sensitive to climatic fluctuations. This proves that the drivers of genetic differentiation in the Southern Ocean are similar to mid and high latitude environments (Maggs et al., 2008). Our study also conforms to a growing body of evidence that population connectivity in marine fish is not driven exclusively by the duration of passive larval dispersal but is influenced by other factors such as the active swimming of adults. It is unclear, however, whether inferred differences

between ecological groups translate into a different potential for local adaptation or extinction, since population connectivity was found to be quite high in all three species and the Pleistocene has not left notable traces on diversification rate (Janko et al., 2011). However, it is notable that most studies have focused on neutral markers in order to interpret the data in demographic rather than locus-specific context. Given that permeability of species barriers differs among the genes (Storchová et al., 2010), it becomes highly important to profit from recent technological and analytical progress in genome and transcriptome analysis (Bernatchez et al., 2011). Comparison of neutral and non-neutral markers should allow to elucidate the potential of local adaptation and speciation in the Southern Ocean.

Acknowledgements

We thank Mark Belchier from the British Antarctic Survey, and the Australian Antarctic Division for providing tissue samples; The Alfred Wegener Institute for logistic support; Bart Hellemans, Šárka Pelikánová and Jana Čechová for invaluable help in laboratory work. This study was supported by: the Belgian Science Policy (BELSPO projects no. EV/01/30B, "PELAGANT" and SD/BA/851, "PADI"); the Grant Agency of Academy of Sciences of the Czech Republic No. KJB600450903 to K.J. and L.Ch.; the Synthesys project conducted at the Muséum National d'Histoire Naturelle (Paris, France), part of the European sixth framework program FR-TAF-325 for K.J.; the Institute of Animal Physiology and Genetics AS CR that receives continuous support from IRP IAPG No. AV0Z50450515. G.E.M was funded by a post-doctoral fellowship provided by the Flemish Fund for Scientific Research (FWO Vlaanderen). J.S. was supported by the Polish Ministry of Science and Higher Education within the program 'Supporting International Mobility of Scientists' and a grant no. NN305376438. J.R. was supported by AFI 6/16 funded by the Natural Environment Resource Council UK. We thank the IPEV program 281 ICOTA coordinated by Philippe Koubbi and Catherine Ozouf-Costaz for samples from Adelie Land.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.margen.2012.05.002.

References

Barnes, D.K.A., De Grave, S., 2000. Biogeography of Southern polar bryozoans. Vie Milieu 50, 261–273.

Bay, L.K., Crozier, R.H., Caley, M.J., 2006. The relationship between population genetic structure and pelagic larval duration in coral reef fishes on the Great Barrier Reef. Mar. Biol. 149, 1247–1256.

Bazin, E., Glémin, S., Galtier, N., 2006. Population size does not influence mitochondrial genetic diversity in animals. Science 312, 570–572.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, F., 2002. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Ge´nome, Populations, Interactions, CNRS UMR 5000. Universite´ de Montpellier II, Montpellier (France).

Bernatchez, L., St-Cyr, J., Normandeau, E., Maes, G.E., Als, T.D., Kalujnaia, S., Cramb, G., Castonguay, M., Hansen, M.M., 2011. Differential timing of gene expression regulation between leptocephali of the two Anguilla eel species in the Sargasso Sea. Ecol. Evol. 1, 459–467.

Bohonak, A.J., 1999. Dispersal, gene flow, and population structure. Q. Rev. Biol. 74, 21–45.

Bonhomme, F., Planes, S., 2000. Some evolutionary arguments about what maintains the pelagic interval in reef fishes. Environ. Biol. Fishes 59, 365–383.

Bradbury, I.R., Laurel, B., Snelgrove, P.V.R., Bentzen, P., Campana, S.E., 2008. Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history. Proc. R. Soc. B. 275, 1803–1809.

Brookfield, J.F.Y., 1996. A simple new method for estimating null allele frequency from heterozygote deficiency. Mol. Ecol. 5, 453–455.

Buonaccorsi, V.P., McDowell, J.R., Graves, J.E., 2001. Reconciling patterns of inter-ocean molecular variance from four classes of molecular markers in blue marlin (*Makaira nigricans*). Mol. Ecol. 10, 1179–1196.

Carreras-Carbonell, J., Macpherson, E., Pascual, M., 2006. Population structure within and between subspecies of the Mediterranean triplefin fish *Tripterygion delaisi* revealed by highly polymorphic microsatellite loci. Mol. Ecol. 15, 3527–3539.

Ciofi, C., Beaumont, M.A., Swingland, I.R., Bruford, M.W., 1999. Genetic divergence and units for conservation in the Komodo dragon *Varanus komodoensis*. Proc. R. Soc. Lond. B Biol. Sci. 266, 2269–2274.

Cornuet, J.M., Luikart, G., 1997. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144, 2001–2014.

Dettai, A., Lautredou, A.-C., Bonillo, C., Goimbault, E., Busson, F., Causse, R., Couloux, A., Cruaud, C., Duhamel, G., Denys, G., Hautecoeur, M., Iglesias, S., Koubbi, P., Lecointre, G., Moteki, M., Pruvost, P., Tercerie, S., Ozouf, C., 2011. The actinopterygian diversity of the CEAMARC cruises: Barcoding and molecular taxonomy as a multi-level tool for new findings. Deep-Sea Res. II Top. Stud. Oceanogr. 58, 250–263.

DeWitt, H.H., Heemstra, P.C., Gon, O., 1990. Nototheniidae. In: Gon, O., Heemstra, P.C. (Eds.), Fishes of the Southern Ocean. JLB Smith Institute of Ichtyology, Grahamstown, pp. 279–331.

de Campos Telles, M.P., Diniz-Filho, J.A.F., 2005. Multiple Mantel tests and isolation-by-distance, taking into account long-term historical divergence. Genet. Mol. Res. 4, 742–748.

Duhamel, G., Kock, K.-H., Balguerias, E., Hureau, J.-C., 1993. Reproduction in fish of the Weddell Sea. Polar Biol. 13, 193–200.

Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620.

Excoffier, Laurent, Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Res. 10, 564–567.

Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479–491.

Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147, 915–925.

Goudet, J., 2001. FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9.3. , http://www.unil.ch/izea/softwares/fstat.html2001.

Hauser, L., Carvalho, G.R., 2008. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish Fish. 9, 333–362.

Hedgecock, D., 1994. Does variance in reproductive success limit effective population sizes of marine organisms? In: Beaumont, A.R. (Ed.), Genetics and evolution of aquatic organisms. Chapman and Hall, London, pp. 122–134.

Hedgecock, D., Barber, P.H., Edmands, S., 2007. Genetic approaches to measuring connectivity. Oceanography 20, 70–79.

Hellberg, M.E., 1998. Sympatric sea shells along the sea's shore: the geography of speciation in the marine gastropod *Tegula*. Evolution 52, 1311–1324.

Hellberg, M.E., 2009. Gene flow and isolation among populations of marine animals. Ann. Rev. Ecol. Evol. Syst. 40, 291–310.

Janko, K., Lecointre, G., DeVries, A., Couloux, A., Cruaud, C., Marshall, C., 2007. Did glacial advances during the Pleistocene influence differently the demographic histories of benthic and pelagic Antarctic shelf fishes? – Inferences from intraspecific mitochondrial and nuclear DNA sequence diversity. BMC Evol. Biol. 7, 220.

Janko, K., Marshall, C., Musilová, Z., Houdt, J.V., Couloux, A., Cruaud, C., Lecointre, G., 2011. Multilocus analyses of an Antarctic fish species flock (Teleostei, Notothenioidei, Trematominae): phylogenetic approach and test of the early-radiation event. Mol. Phylogenet. Evol. 60, 305–316.

Kock, K.-H., Pshenichnov, L.K., Devries, A.L., 2006. Evidence for egg brooding and parental care in icefish and other notothenioids in the Southern Ocean. Antarct. Sci. 18, 223–227.

Kojima, S., Segawa, R., Hayashi, I., 1997. Genetic differentiation among populations of the Japanese turban shell *Turbo* (*Batillus*) *cornutus* corresponding to warm currents. Mar. Ecol. Prog. Ser. 150, 149–155.

Kuhner, M.K., 2003. LAMARC: estimating population genetic parameter from molecular data. In: Salemi, M., Vandamme, A.M. (Eds.), The Phylogenetic Handbook; a practical approach to DNA and protein phylogeny. Cambridge University Press.

Kuhner, M.K., Yamato, J., Felsenstein, J., 1998. Maximum likelihood estimation of population growth rates based on the coalescent. Genetics 149, 429–434.

La Mesa, Mario, Ascoli, L., Caputo, V., 2006. Gametogenesis and reproductive strategies in some species of the Antarctic fish genus *Trematomus* (Nototheniidae) from Terra Nova Bay, Ross Sea. Polar Biol. 29, 963–970.

Larmuseau, M.H.D., Raeymaekers, J.A.M., Hellemans, B., Van Houdt, J.K., Volckaert, F.A.M., 2010. Mito-nuclear discordance in the degree of population differentiation in a marine goby. Heredity 105, 532–542.

Lautredou, A.-C., Bonillo, C., Denys, G., Cruaud, C., Ozouf-Costaz, C., Lecointre, G., Dettai, A., 2010. Molecular taxonomy and identification within the Antarctic genus Trematomus (Notothenioidei, Teleostei): How valuable is barcoding with COI? Polar Sci. 4, 333–352.

Leis, J.M., 2006. Are larvae of demersal fishes plankton or nekton? Adv. Mar. Biol. 51, 57–141.

Lessa, E.P., Cook, J.A., Patton, J.L., 2003. Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. Proc. Nat. Acad. Sci. U. S. A. 100, 10031–10034.

Librado, P., Rozas, J., 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.

Lowe, W.H., Allendorf, F.W., 2010. What can genetics tell us about population connectivity? Mol. Ecol. 19, 3038–3051.

Maggs, C.A., Castilho, R., Foltz, D., Henzler, C., Jolly, M.T., Kelly, J., Olsen, J., Perez, K.E., Stam, W., Väinölä, R., Viard, F., Wares, J., 2008. Evaluating signatures of glacial refugia in North Atlantic benthic marine taxa. Ecol 89, 108–122.

Matschiner, M., Hanel, R., Salzburger, W., 2009. Gene flow by larval dispersal in the Antarctic notothenioid fish *Gobionotothen gibberifrons*. Mol. Ecol. 18, 2574–2587.

McGaughran, A., Torricelli, G., Carapelli, A., Frati, F., Stevens, M.I., Convey, P., Hogg, I.D., 2010. Contrasting phylogeographical patterns for springtails reflect different

evolutionary histories between the Antarctic Peninsula and continental Antarctica. J. Biogeo 37, 103–119.
O'Reilly, P.T., Canino, M.F., Bailey, K.M., Bentzen, P., 2004. Inverserelationship between F-ST and microsatellite polymorphism in the marine fish, walleye pollock (Theragra chalcogramma): implications for resolving weak population structure. Mol. Ecol. 13, 1799–1814.
Orsi, A.H., Whitworth, T., Nowlin, W.D., 1995. On the meridional extent and fronts of the Antarctic Circumpolar Current. Deep Sea Res. Part I 42, 641–673.
Papetti, C., Susana, Emanuele, La Mesa, M., Kock, K.-H., Patarnello, T., Zane, L., 2007. Microsatellite analysis reveals genetic differentiation between year-classes in the icefish *Chaenocephalus aceratus* at South Shetlands and Elephant Island. Polar Biol. 30, 1605–1613.
Papetti, C., Susana, E., Patarnello, T., Zane, L., 2009. Spatial and temporal boundaries to gene flow between *Chaenocephalus aceratus* populations at South Orkney and South Shetlands. Mar. Ecol. Prog. Ser. 376, 269–281.
Papetti, C., Pujolar, J.M., Mezzavilla, M., La Mesa, M., Rock, J., Zane, L., Patarnello, T., 2012. Population genetic structure and gene flow patterns between populations of the Antarctic icefish *Chionodraco rastrospinosus*. J. Biogeogr.
Patarnello, T., Marcato, S., Zane, L., Varotto, V., Bargelloni, L., 2003. Phylogeography of the *Chionodraco* genus (Perciformes, Channichthyidae) in the Southern Ocean. Mol. Phylogenet. Evol. 28, 420–429.
Patarnello, T., Verde, C., di Prisco, G., Bargelloni, L., Zane, L., 2011. How will fish that evolved at constant sub-zero temperatures cope with global warming? Notothenioids as a case study. Bioessays 33, 260–268.
Planes, S., Parroni, M., Chauvet, C., 1998. Evidence of limited gene flow in three species of coral reef fishes in the lagoon of New Caledonia. Mar. Biol. 130, 361–368.
Posada, D., 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Purcell, J.F.H., Cowen, R.K., Hughes, R.C., Williams, D.A., 2006. Weak genetic structure indicates strong dispersal limits: a tale of two coral reef fish. Proc. R. Soc. B. 273, 1483–1490.
Ramos-Onsins, S.E., Rozas, J., 2002. Statistical properties of new neutrality tests against population growth. Mol. Biol. Evol. 19, 2092–2100.
Rico, C., Turner, G.F., 2002. Extreme microallopatric divergence in a cichlid species from Lake Malawi. Mol. Ecol. 11, 1585–1590.
Rieseberg, L.H., Burke, J.M., 2001. A genic view of species integration. J. Evol. Biol. 14, 883–886.
Ritchie, P.A., Millar, C.D., Gibb, G.C., Baroni, C., Lambert, D.M., 2004. Ancient DNA enables timing of the Pleistocene origin and Holocene expansion of two Adélie Penguin lineages in Antarctica. Mol. Biol. Evol. 21, 240–248.
Rogers, A.D., 2007. Evolution and biodiversity of Antarctic organisms: a molecular perspective. Philos. Trans. R. Soc. Lond. B Biol. Sci. 362, 2191–2214.
Rogers, A.D., 2012. Evolution and biodiversity of Antarctic organisms: a molecular perspective. In: Rogers, A.D., Johnston, N.M., Murphy, E.J., Clarke, A. (Eds.), Antarctic Ecosystems: An Extreme Environment in a Changing World. Blackwell Publishing Ltd.
Rousset, F., 2008. Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol. Ecol. Res. 8, 103–106.
Rutschmann, S., Matschiner, M., Damerau, M., Muschick, M., Lehmann, M.F., Hanel, R., Salzburger, W., 2011. Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation. Mol. Ecol. 20, 4707–4721.
Ruzzante, D.E., Mariani, S., Bekkevold, D., André, C., Mosegaard, H., Clausen, L.A.W., Dahlgren, T.G., Hutchinson, W.F., Hatfield, E.M.C., Torstensen, E., Brigham, J., Simmonds, E.J., Laikre, L., Larsson, L.C., Stet, R.J.M., Ryman, N., Carvalho, G.R., et al., 2006. Biocomplexity in a highly migratory pelagic marine fish, Atlantic herring. Proc Biol Sci 273 (1593), 1459–1464.
Ryman, N., Palm, S., 2006. POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. Mol. Ecol. Notes 6, 600–602.
Selkoe, K.A., Henzler, C.M., Gaines, S.D., 2008. Seascape genetics and the spatial ecology of marine populations. Fish Fish. 9, 363–377.
Shaw, P.W., Arkhipkin, A.I., Al-Khairulla, H., 2004. Genetic structuring of Patagonian toothfish populations in the Southwest Atlantic Ocean: the effect of the Antarctic Polar Front and deep-water troughs as barriers to genetic exchange. Mol. Ecol. 13, 3293–3303.
Skillings, D.J., Bird, C.E., Toonen, R.J., 2011. Gateways to Hawaii: Genetic Population Structure of the Tropical Sea Cucumber *Holothuria atra*. J. Mar. Biol. Article ID 783030 16 pages.
Slatkin, M., 2005. Seeing ghosts: the effect of unsampled populations on migration rates estimated for sampled populations. Mol. Ecol. 14, 67–73.
Storchová, R., Reif, J., Nachman, M.W., 2010. Female heterogamety and speciation: reduced introgression of the Z chromosome between two species of nightingales. Evolution 64, 456–471.
Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585–595.
Taylor, M.S., Hellberg, M.E., 2003. Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science 299, 107–109.
Thatje, S., Hillenbrand, C.-D., Mackensen, A., Larter, R., 2008. Life hung by a thread: endurance of antarctic fauna in glacial periods. Ecology 89, 682–692.
Thorrold, S.R., Zacherl, D.C., Levin, L.A., 2007. Population connectivity and larval dispersal : using geochemical signatures in calcified structures. Oceanography 20 (3), 80–89.
Thorson, G., 1950. Reproductive and larval ecology of marine bottom invertebrates. Biol. Rev. 25, 1–45.
Van de Putte, A.P., Van Houdt, J.K., Maes, G.E., Janko, K., Koubbi, P., Rock, J., Volckaert, F.A.M., 2009. Species identification in the trematomid family using nuclear genetic markers. Polar Biol. 32, 1731–1741.
Van de Putte, A.P., Van Houdt, J.K.J., Maes, G.E., Hellemans, B., Collins, M.A., Volckaert, F.A.M., 2012. High genetic diversity and connectivity in a common mesopelagic fish of the Southern Ocean: the myctophid *Electrona antarctica*. Deep-Sea Res. II Top. Stud. Oceanogr. 59–60, 199–207.
Van Houdt, J.K.J., Hellemans, B., Van De Putte, A., Koubbi, P., Volckaert, F.A.M., 2006. Isolation and multiplex analysis of six polymorphic microsatellites in the Antarctic notothenioid fish, *Trematomus newnesi*. Mol. Ecol. Notes 6, 157–159.
Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4, 535–538.
Volckaert, F.A.M., Rock, J., Van de Putte, A.P., 2012. Connectivity and Molecular Ecology in Antarctic Fish. In: di Prisco, G., Verde, C. (Eds.), Pole to Pole, Adaptation And Evolution In Marine Environments, vol. 1 (5). Springer Berlin, Heidelberg, pp. 75–96.
Waples, R.S., 1987. A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41, 385–400.
Weir, B.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. Evolution 38, 1358–1370.
Zane, L., Marcato, S., Bargelloni, L., Bortolotto, E., Papetti, C., Simonato, M., Varotto, V., Patarnello, T., 2006. Demographic history and population structure of the Antarctic silverfish *Pleuragramma antarcticum*. Mol. Ecol. 15, 4499–4511.