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Phylogeography of the *Rhabditis* (*Pellioditis*) *marina* species complex: evidence for long-distance dispersal, and for range expansions and restricted gene flow in the northeast Atlantic

S. DERYCKE,*‡ T. REMERIE,*‡ T. BACKELJAU,§¶ A. VIERSTRAETE,†‡ J. VANFLETEREN,†‡ M. VINCX* and T. MOENS*

Department of Biology, Marine Biology Section, Ghent University, Krijgslaan 281, S8, 9000 Ghent, Belgium, †Department of Biology, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium, ‡CeMoFE, Ghent University, K.L. Ledeganckstraat 35, 9000 Ghent, Belgium, §Royal Belgian Institute of Natural Sciences, Vautierstraat 29, 1000 Brussels, Belgium, ¶Evolutionary Biology Group, Department of Biology, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium

Abstract

Pinpointing processes that structure the geographical distribution of genetic diversity of marine species and lead to speciation is challenging because of the lack of obvious dispersal barriers and the likelihood of substantial (passive) dispersal in oceans. In addition, cryptic radiations with sympatric distributions abound in marine species, challenging the allopatric speciation mechanism. Here, we present a phylogeographical study of the marine nematode species complex *Rhabditis* (*Pellioditis*) *marina* to investigate processes shaping genetic structure and speciation. *Rhabditis* (*P.*) *marina* lives on decaying macroalgae in the intertidal, and may therefore disperse over considerable distances. *Rhabditis* (*P.*) *marina* consists of several cryptic species sympatrically distributed at a local scale. Genetic variation in the COI gene was screened in 1362 specimens from 45 locations around the world. Two nuclear DNA genes (ITS and D2D3) were sequenced to infer phylogenetic species. We found evidence for ten sympatrically distributed cryptic species, seven of which show a strong genetic structuring. A historical signature showed evidence for restricted gene flow with occasional long-distance dispersal and range expansions pre-dating the last glacial maximum. Our data also point to a genetic break around the British Isles and a contact zone in the Southern Bight of the North Sea. We provide evidence for the transoceanic distribution of at least one cryptic species (PmIII) and discuss the dispersal capacity of marine nematodes. The allopatric distribution of some intraspecific phylogroups and of closely related cryptic species points to the potential for allopatric speciation in *R.* (*P.*) *marina*.

Keywords: cosmopolitanism, cryptic species, dispersal, nematode, phylogeography, *R.* (*P.*) *marina*

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Introduction

The Quaternary climate changes have influenced the distribution and radiation of temperate terrestrial (Taberlet *et al*. 1998) and marine species (Avise 2000). Alterations of glacial and interglacial periods have resulted in the contraction, expansion and fragmentation of species ranges leading to population bottlenecks and alterations of gene flow. Intuitively, drastic changes in connectivity might have driven species to extinction (e.g. Scott *et al*. 2007) and may have produced new diversity (Ribera & Vogler 2004; Turgeon *et al*. 2005). Pinpointing the processes that lead to diversification in the marine environment is challenging because of the lack of obvious barriers to gene flow coupled with the large dispersal abilities of many pelagic and planktonic species and the prevalence of a high degree of cryptic diversity (Knowlton 2000) often found in sympatry (Dawson *et al*. 2002). Explanations for marine speciation have concomitantly shifted from a focus on broad-scale allopatric speciation to mechanisms that may be situated at much finer geographical scales (Taylor & Hellberg 2005). Phylogeographical studies provide an excellent way to investigate the effects of changes in geographical distributions

Correspondence: Sofie Derycke, Fax: 09 264 85 98; E-mail: s.derycke@UGent.be

on intraspecific genetic diversity, and have highlighted a significant structuring of marine populations (Barber *et al*. 2000; Perrin *et al*. 2004), even in species with pelagic larval stages (Taylor & Hellberg 2003). This genetic structuring may ultimately lead to speciation.

Despite a ubiquitous distribution of marine nematodes around the world, phylogeographical studies hitherto only dealt with terrestrial/parasitic species (Plantard & Porte 2004; Nieberding *et al*. 2005). Global nematode diversity estimates range between 10^5–10^8 (morphological) species (Coomans 2000; Lambshead & Boucher 2003). For marine environments, nematodes typically are the dominant taxon with densities ranging between 10^5–10^8 individuals/m^2 (Heip *et al*. 1985), and with several tens of species co-occurring at a local scale. This high numerical dominance and species diversity at a local scale raise questions on which processes are responsible for speciation in this phylum. Here, we use the phylogeographical history of the marine nematode *Rhabditis* (*Pellioditis*) *marina* Bastian 1865 to infer the importance of microevolutionary processes mediating distribution and speciation in the marine environment. *Rhabditis* (*P.*) *marina* inhabits macroalgae in the intertidal zone of coasts and estuaries around the world, including Arctic, Antarctic and tropical areas, suggesting tolerance to a wide temperature range. It abounds on decomposing macroalgae that are washed ashore, and may therefore be dispersed over considerable distances. Reproduction is obligately heterosexual. Females can be oviparous or ovoviviparous and have a high reproductive output (up to 600 eggs/female under optimal conditions, Vranken & Heip 1983). The generation time is very short and strongly temperature-dependent, ranging from 2 to 7 days at temperatures of 25 °C and 9 °C, respectively (Moens & Vincx 2000). Juveniles moult four times and can form a metabolically less active dauer stage under conditions of crowding and food (= bacteria) limitation.

At first glance, nematode morphology seems simple, so that the detection of most diagnostic features often requires high-resolution optics and expertise. Recently, substantial cryptic diversity was discovered in *R.* (*P.*) *marina* (Derycke *et al*. 2005, 2008) and in another geographically widespread marine nematode species (Derycke *et al*. 2007a) supporting the contention that so-called cosmopolitan species may actually consist of series of cryptic species. In contrast, evidence for cosmopolitanism based on molecular data has been found in other small eukaryotic organisms with problematic morphology (Darling *et al*. 2000; Finlay *et al*. 2006) and recently, in the marine nematode *Terschellingia longicaudata* (Bhadury *et al*. 2008). In the case of *R.* (*P.*) *marina*, seven molecular lineages have been identified, each of which turned out to be morphometrically well-differentiated (Derycke *et al*. 2008). They showed amounts of molecular differentiation comparable to those observed among other, well-known rhabditid species, and breeding experiments between two closely related lineages showed that they were reproductively isolated (Derycke *et al*. 2008). To date, none of these lineages have been formally described.

Long-distance dispersal of parasitic terrestrial nematodes, most likely through transport of cysts, has been suggested (Plantard & Porte 2004), but data for free-living marine nematodes are hitherto restricted to studies at small geographical scales in Belgium and the Netherlands (100 km, Derycke *et al*. 2005, 2006, 2007a). At this scale, *R.* (*P.*) *marina* showed restricted gene flow indicating that dispersal through rafting is limited (Derycke *et al*. 2006). The genetic structuring at the local scale may have been confounded, however, by colonization–extinction dynamics typical of ephemeral populations (Derycke *et al*. 2007b).

The present study aims to identify pan-European and global patterns of genetic diversity in the *R.* (*P.*) *marina* species complex by adding 36 new locations from the Atlantic and Pacific Ocean, and from the Mediterranean and the Baltic Sea to our previous small-scale data set. The *R.* (*P.*) *marina* complex forms an excellent model to investigate the extent of cosmopolitanism and dispersal abilities of marine nematodes. Because of the cryptic diversity and restricted gene flow patterns we previously observed at local scales in Belgium and the Netherlands, we expected at macrogeographical scales to find (i) many more new cryptic species, and (ii) a strong genetic structure within each species. Based on other phylogeographical studies in marine organisms from the northeast Atlantic, we expected to find (i) a lower genetic diversity at higher latitudes, and (ii) signatures of historical expansions in northern areas due to Pleistocene geographical changes.

Materials and methods

Sampling and genetic analysis

From June 2005 until April 2007, *Rhabditis* (*Pellioditis*) *marina* were collected from numerous localities throughout Europe (7 Baltic, 1 Kattegat, 12 Atlantic and 9 Mediterranean localities), northeast America (3), Canada (1), Mexico (1), South Africa (1) and Australia (1) (Table 1). Specimens of *R.* (*P.*) *marina* were isolated from decaying seaweeds and/or seagrasses washed ashore in the intertidal zone. The organic material was collected from one or more piles per location and incubated on marine agar dishes, allowing nematodes to move into the agar. Using a stereomicroscope, *R.* (*P.*) *marina* were then handpicked from the agar and preserved in acetone until molecular processing. In this way, 741 specimens were collected from 36 localities worldwide. We also added published data on *R.* (*P.*) *marina* from Derycke *et al*. (2005, 2006). We used haplotype frequencies from Derycke *et al*. (2006) from localities with $n > 5$ and averaged them over seasons. Our total data set thus comprised 1362 specimens from 45 localities (Table 1).

Table 1 *Rhabditis* (*Pellioditis*) *marina*. Listing of the sampled populations with their waterbasin, country and location. Codes used for each location are mentioned, together with latitutidal and longitudinal degrees. The total number of sampled specimens (*n*) along with the number of specimens belonging to each species are indicated. Haplotype diversity (*h*) and the standard error are also shown

						PmI		PmII		PmIIIb		PmIIIa		PmIV		PmV		PmVI		PmVII		PmVIII		PmIX		PmX		
Waterbasin	Country	Location	Code	Latitude	Longitude	*n*	*h* (SD)	*n*	*h* (SD)	*n*	*h* (SD)	*n*	*h* (SD)	*n*	*h* (SD)	*n*	*h* (SD)	*n*	*h* (SD)	*n*	*h* (SD)	*n*	*h* (SD)	*n*	*h* (SD)	*n*	*h* (SD)	*n*
Baltic Sea	Germany	Rostock	Ros	54°11′N	12°08′E			17	0.71 (0.11)																			17
Baltic Sea	Germany	Kiel	Ki	54°19′N	10°08′E			1	–							1	–											2
Baltic Sea	Poland	Isle of Rugia	Ru	54°30′N	13°24′E			9	0.22 (0.17)																			9
Baltic Sea	Poland	Hel	He	54°36′N	18°48′E			4	–							10	0.69 (0.10)											14
Baltic Sea	Poland	Kuznica	Ku	Ku 54°43′N	18°35′E			19	0.49 (0.10)																			19
Baltic Sea	Germany	Flensburg	Fle	54°46′N	9°26′E			21	0.65 (0.07)																			21
Kattegat	Sweden	Strömstad	swe	58°53′N	11°07′E					15	0.76 (0.09)																	15
North Sea	Germany	Sylt	Sy	55°01′N	8°26′E			9	0.42 (0.19)							10	0.71 (0.12)											19
North Sea	Belgium	Nieuwpoort	Ni	51°09′N	2°43′E	44	0.84 (0.02)	2	–	16	60.43 (0.13)																	62
North Sea	Belgium	Blankenberge*	Bl	51°19′N	3°8′E	27	0.51 (0.11)	28	0.87 (0.05)	24	0.68 (0.04)							5	0.00 (0.00)							2	0.00 (0.00)	86
North Sea	The Netherlands	Paulina*	Pa	51°21′N	3°49′E	43	0.87 (0.03)	17	0.83 (0.05)	12	0.62 (0.09)																	72
North Sea	The Netherlands	Kruispolderhaven*	Kr	51°22′N	4°3′E	48	0.67 (0.04)																					48
North Sea	The Netherlands	Zeedorp*	Ze	51°24′N	3°58′E	43	0.78 (0.05)			32	0.74 (0.05)																	75
North Sea	The Netherlands	Breskens*	Br	51°24′N	3°33′E	49	0.68 (0.06)																					49
North Sea	The Netherlands	Sloehaven*	Sl	51°27′N	3°36′E	41	0.79 (0.04)	17	0.23 (0.13)	36	0.70 (0.05)																	94
North Sea	The Netherlands	Oosterschelde*	Os	51°36′N	3°50′E	46	0.63 (0.07)			17	0.68 (0.06)																	63
North Sea	The Netherlands	Lake Grevelingen–Brouwershaven*	GrB	51°44′N	3°57′E			24	0.87 (0.05)	15	0.56 (0.01)																	72
North Sea	The Netherlands	Lake Grevelingen-Scharendijke	GrS	51°54′N	3° 49′E																							29
North Sea	North Scotland	Westroy	Sc	59°17′N	2°57′W	10	0.47 (0.13)	15	0.69 (0.07)					33	0.62 (0.06)													25
North Sea	Norway	Aurlandsvangen	No	60°54′N	7°10′E			29	0.42 (0.11)					29	0.64 (0.07)													29
English Channel	France	St Malo	Ma	48°38′N	2°01′W			39	0.75 (0.02)																			39
English Channel	France	Roscoff	Ro	48°43′ N	3°59′W	1	–							1	–					21	0.83 (0.06)							23
English Channel	Great Britain	Plymouth	Pl	50°22′N	4°9′E			31	0.74 (0.04)																			31
NE Atlantic	Portugal	Tavira	Po	37°07′N	7°38′W											23	0.68 (0.08)											23
NE Atlantic	Spain	San Pedro	San	43°23′N	8°17′W									10	0.00 (0.00)													10
NE Atlantic	France	Vaux sur mer	Va	45°38′N	1°04′W			3	–	16	16 0.00 (0.00)																	19
NE Atlantic	France	Sables d′ Olonne	Sa	46°29′N	1°46′W			10	0.78 (0.09)									14	0.82 (0.06)									24
NE Atlantic	Ireland	Cork	Co	51°53′N	8°23′W									5	0.80 (0.16)					2	0.00 (0.00)							7
NE Atlantic	Great Britain	Wales	Wa	53°24′N	4°19′W			11	0.00 (0.00)																			11
Mediterranean	Cyprus	Larnaca	La	34°58′N	33°41′E													18	0.82 (0.06)									18
Mediterranean	Greece	Crete	GrC2	35°19′N	25°14′E																	17	0.77 (0.07)					17
Mediterranean	Greece	Crete	GrC	35°19′N	25°22′E													3	0.00 (0.00)			22	0.56 (0.10)					25
Mediterranean	Spain	Alicante	Ali	38°40′N	0°07′E											2	–											2
Mediterranean	Spain	Palamos	Pal	41°50′N	3°07′E																	29	0.58 (0.04)					29
Mediterranean	Croatia	Mljet Islands	CrM	42°44′N	17°32′E																	19	0.72 (0.08)					19
Mediterranean	Greece	Lagonissi	GrL	42°44′N	25°53′E											22	0.80 (0.06)											22
Mediterranean	France	St Aygulf	Ay	43°23′N	6°43′E											11	0.76 (0.08)											11
Mediterranean	Croatia	Brodarica	CrB	43°40′N	15°55′E																	28	0.60 (0.06)					28
NW Atlantic	Mexico	Yucatan	Me	21°12′N	87°48′W																			21	0.75 (0.06)			21
NW Atlantic	USA	Florida	Fl	27°15′N	82°31′W					28	0.52 (0.03)																	28
NW Atlantic	USA	New York	NY	40°42′N	74°00′W					28	0.65 (0.05)																	28
NW Atlantic	USA	Boston	Bo	42°21′N	71°03′W					30	0.56 (0.09)																	30
NW Atlantic	celand	Prestbakki	Ic	65°19′N	21°13′W	20	0.50 (0.12)							1	–													21
Indian Ocean	South Africa	Transkei	Af	31°45′S	29°22′E													7	0.00 (0.00)							21	0.00 (0.00)	28
NE Pacific	Canada	Vancouver Island	Ca	39°35′N	125°50′W																							1
SW Pacific	Queensland	Cairns	Au	16°55′S	145°45′E							27	0.52 (0.03)															27
		TOTAL				372		306		269		27		79		79		47		23		115		21		23		1362

*averaged across seasons (data from Derycke *et al*. 2006).

A fragment of the mitochondrial cytochrome oxidase c subunit 1 (COI) gene was amplified and analysed according to Derycke *et al*. (2005). In short, DNA was extracted from single nematodes and 1 μL was used for polymerase chain reaction (PCR) with primers JB3 and JB5, yielding PCR products of 426 bp long. These were screened for genetic variation with the single-strand conformation polymorphism (SSCP) method. Each location was analysed independently, and all differing SSCP banding profiles were sequenced with the aforementioned primers. The most common band profile in each location was sequenced twice. For all new populations, identical SSCP profiles yielded identical sequences. In total, 310 individuals were sequenced for the COI region. All new *R.* (*P.*) *marina* sequences (88) are available in GenBank under accession nos AM937121–AM937225. Trimming the primer sites from the sequenced PCR product resulted in fragments of 396 bp long.

In a subset of specimens (n = 1–8) of each mitochondrial DNA (mtDNA) lineage, we amplified two nuclear DNA (nucDNA) gene regions: the internal transcribed spacer region (ITS1–5.8S–ITS2) and the D2D3 region of the ribosomal large subunit. Details of amplification and accession numbers can be found in Derycke *et al*. (2005, 2008). The D2D3 data set consisted of 53 *R.* (*P.*) *marina* sequences, 25 of which were new to this study. Of these 25 new sequences, 8 were different (AM937033–AM937040). The ITS data set had 48 *R.* (*P.*) *marina* sequences, 20 of which were new to this study. Of these, 13 sequences were different (AM937041–AM937053).

Data analysis

Phylogenetic analysis. COI sequences were aligned in CLUSTAL_X version 1.81 (Thompson *et al*. 1997) using default gap opening/extension costs of 15/6.66. Sequences of the closely related species *Rhabditis* (*Rhabditis*) *nidrosiensis* and *Rhabditis* (*Pellioditis*) *mediterranea* obtained from Derycke *et al*. (2008) were added to the data set. All 156 haplotypes were easily alignable and no indels were observed. Sequences were blasted in GenBank and translated in MEGA version 3.1 (Kumar *et al*. 2004) to ensure sequence integrity.

The ITS and D2D3 sequences were concatenated and aligned in CLUSTAL_X version 1.81. The alignment contained several indels, mainly in the ITS part, and was screened for unreliable positions in SOAP 1.2a4 (Löytynoja & Milinkovitch 2001) using the following CLUSTAL W parameter range: gap penalties were allowed to range from 11 to 19 with a two-step increase, and extension penalties ranged between 3 and 11, also with a two-step increase. The default parameter settings in CLUSTAL_X were chosen for the reference alignment. The unreliability of each position is determined by the proportion of nonreference alignments that differ, for that position, from the reference alignment (Löytynoja & Milinkovitch 2001). Unreliable positions (211 out of 1577) were removed for phylogenetic analyses.

Trees were rooted with the congener *Rhabditis* (*Rhabditis*) *nidrosiensis*, which often co-occurs with *R.* (*P.*) *marina* on decomposing macroalgae. At this point, no COI data is available for other *Rhabditis* species. Mean sequence divergence within and between haplotype groups was calculated in MEGA version 3.1 using the *P*-distance model. The net sequence divergences (= intraspecific sequence variability of each of two species subtracted from the mean sequence divergence between these two species) were calculated using *P*-distances in MEGA.

The evolutionary model that best fitted the mtDNA and nucDNA sequences was determined with MODELTEST 3.7 (Posada & Crandall 1998) using the Akaike information criterion (AIC). The HKY + I + G and GTR + I + G model best fitted the mtDNA and nucDNA data, respectively. Most parsimonious trees (MP) were calculated using heuristic searches and a tree-bisection–reconnection branch swapping algorithm (10 000 rearrangements) with random stepwise addition of sequences in 100 replicate trials in PAUP* 4.0 beta 10 (Swofford 1998). Robustness of the MP and neighbour-joining (NJ) trees was tested by bootstrapping with 1000 replications and 10 replicate trials of sequence addition. In addition, a Bayesian analysis was performed in MRBAYES version 3.1.2 (Huelsenbeck & Ronquist 2005). Four independent Markov chains were run for 31 000 000 (mtDNA) and 20 000 000 (nucDNA) generations, with a tree saved every 10 000th generation. The first 200 (mtDNA) and 300 (nucDNA) trees were discarded as burn-in.

Phylogeographical and genetic structure analyses. Haplotype diversity (h) and its standard deviation were calculated for each species in each locality with ARLEQUIN 3.0 (Schneider *et al*. 2000) (Table 1). Patterns of genetic structuring among geographical localities (n > 5) for each species were estimated using ARLEQUIN 3.0. Population pairwise Φ_{ST} values were calculated using Tamura–Nei distances (Tamura & Nei 1993, Appendix I), as the Hasegawa–Kishino–Yano (HKY) model is not implemented in ARLEQUIN 3.0. An analysis of molecular variance (AMOVA) was used to investigate the percentage of variation within and between locations (and groups of populations). Significance levels were determined with 1000 permutations and were corrected for multiple comparisons. Due to small sample sizes and/or too few available populations, population pairwise Φ_{ST} values and AMOVA were not performed for the phylogenetic species PmVII, PmIX and PmX (Pm, *Pellioditis marina*). For the four most abundant phylogenetic species (PmI, PmII, PmIII and PmVIII), additional geographical structuring was investigated with a spatial analysis of molecular variance using SAMOVA 1.0 (Dupanloup *et al*. 2002). This procedure defines groups of populations that are geographically homogeneous and that are maximally differentiated from each other.

Demographic analyses. A haplotype network was constructed for each species using the statistical parsimony procedure in the program TCS version 1.18 (Clement *et al*. 2000). Haplotypes were connected at the 95% confidence level and loops in the network were resolved according to the recommendations of Crandall & Templeton (1993). Tajima's *D* (Tajima 1989) and Fu's *Fs* (Fu 1997) neutrality tests were performed to infer whether sequence evolution in the lineages was neutral and whether geographical groups showed signs of deviations from neutrality. When both neutrality tests yielded test statistics that were significantly different from zero, the frequency distribution of pairwise sequence differences was analysed by mismatch analysis and compared to the expected distribution under a sudden expansion model using 100 bootstrap replicates and quantifying the sum of squared deviations between observed and expected distributions (Rogers & Harpending 1992). This model of sudden expansion assumes (i) an infinite site mutation model, (ii) a panmicitic population, and (iii) the occurrence of a single sudden expansion, but the model still stands even when the mutational process is poorly understood, and when populations are not panmictic (Rogers *et al*. 1996). We used the mismatch analysis solely to investigate whether the species have experienced an expansion. Historical demographic changes were also investigated using the generalized skyline plot (Strimmer & Pybus 2001). This method assumes a clock-like evolution and does not account for phylogenetic error. The results of the method should therefore be regarded as rough estimates, rather than as absolute values. To make the generalized skyline plot, maximum-likelihood (ML) trees were estimated with the HKY + *I* + *G* model (see above) and a molecular-clock assumption in PAUP* 4.0 beta 10. Clock-like evolution could not be rejected using the likelihood-ratio test. The generalized skyline plot was generated from the ML trees in GENIE 3.0 (Pybus & Rambaut 2002) using the smoothing parameter ε. The AICc estimate of ε was very low (–1.69 E-8) and the generalized skyline plot contained a few gaps and pikes. We therefore set ε = 0.0001.

Results

Phylogenetic analyses

The screening of the COI gene in 741 specimens collected from around the world yielded 107 haplotypes, 92 of which had not been previously reported. The complete *Rhabditis* (*Pellioditis*) *marina* data set, including the data of Derycke *et al*. (2006), comprised 1365 screened specimens and 155 haplotypes. The NJ tree showed that three sequences (two from Florida and one from Mexico) formed a monophyletic group that was highly divergent from all other rhabditid sequences (data not shown). These potentially misidentified nematodes were removed from the data set. One sequence from Rostock was pooled with *Rhabditis* (*Rhabditis*) *nidrosiensis* (Fig. 1a) and was also removed from the data set for all subsequent analyses. The 396-bp long fragment of *R.* (*P.*) *marina* contained 132 (33.3%) variable sites, 103 of which were parsimony informative. Not all substitutions were synonymous, and the amino acid alignment yielded 11 variable sites (8.3%). The 1366-bp long nucDNA fragment contained 519 variable sites, 318 of which were parsimony informative.

Phylogenetic analyses of the mtDNA and nucDNA genes identified ten highly supported, monophyletic clades (PmI–PmX) (Fig. 1). The mtDNA clades PmII and PmVI were not well supported, but were highly supported in the nucDNA trees and both lineages also have quite distinct morphologies (Derycke *et al*. 2008). Divergence between PmII and PmVI and their closest relatives was also higher than the intraspecific values. Mitochondrial DNA haplotypes within each of the ten lineages were very similar (maximum intraspecific divergence 0.5–5.2%, average divergence between the intraspecific phylogroups 1.6–4.3%), while haplotypes from different lineages were separated by at least 17 substitutions (average interspecific divergence 4.6–11.7%, Table 2). Each mtDNA clade is therefore treated as a different phylogenetic species. Some substructuring within the mtDNA clades PmIII, PmIV, PmV and PmVI was present, which was absent in the nucDNA trees (Fig. 1b). We treat these subclades as intraspecific variation. Deeper phylogenetic relationships were well resolved in the nucDNA trees (Fig. 1b): PmI appeared as sistergroup of PmIV, while PmII and PmX, PmVII and PmVIII, and PmVI and PmIX formed three other pairs of sister taxa. In addition, PmV was more closely related to PmVI-PmIX, and PmIII to PmVII-PmVIII. *Rhabditis* (*Pellioditis*) *mediterranea* was placed in the ingroup, although its position depended on the markers used. This suggests that it represents still another species in the Pm species complex (Sudhaus & Nimrich 1989). The taxonomic status of the Z3 branch has been discussed in Derycke *et al*. (2008) and because of the limited number of specimens and data on this branch, we do not treat it further here. The single individual from Canada did not belong to any of the ten species.

Phylogeographical and genetic structure analyses

The distribution patterns and abundances differ widely across the ten *R.* (*P.*) *marina* species (Table 1, Fig. 2). PmI was restricted to the northeast Atlantic and dominated the North Sea, while PmII showed a continuous range from the Bay of Biscay into the Baltic Sea. PmIII was frequently observed in the North Sea, and dominated the east coast of North America. As such, PmIII showed a transatlantic distribution range. Of all specimens analysed, 70% belonged to these three species. PmVIII was the next most frequently encountered species (8% of all specimens analysed) and was restricted to the Mediterranean Sea. In fact, only three species have been found in our Mediterranean samples

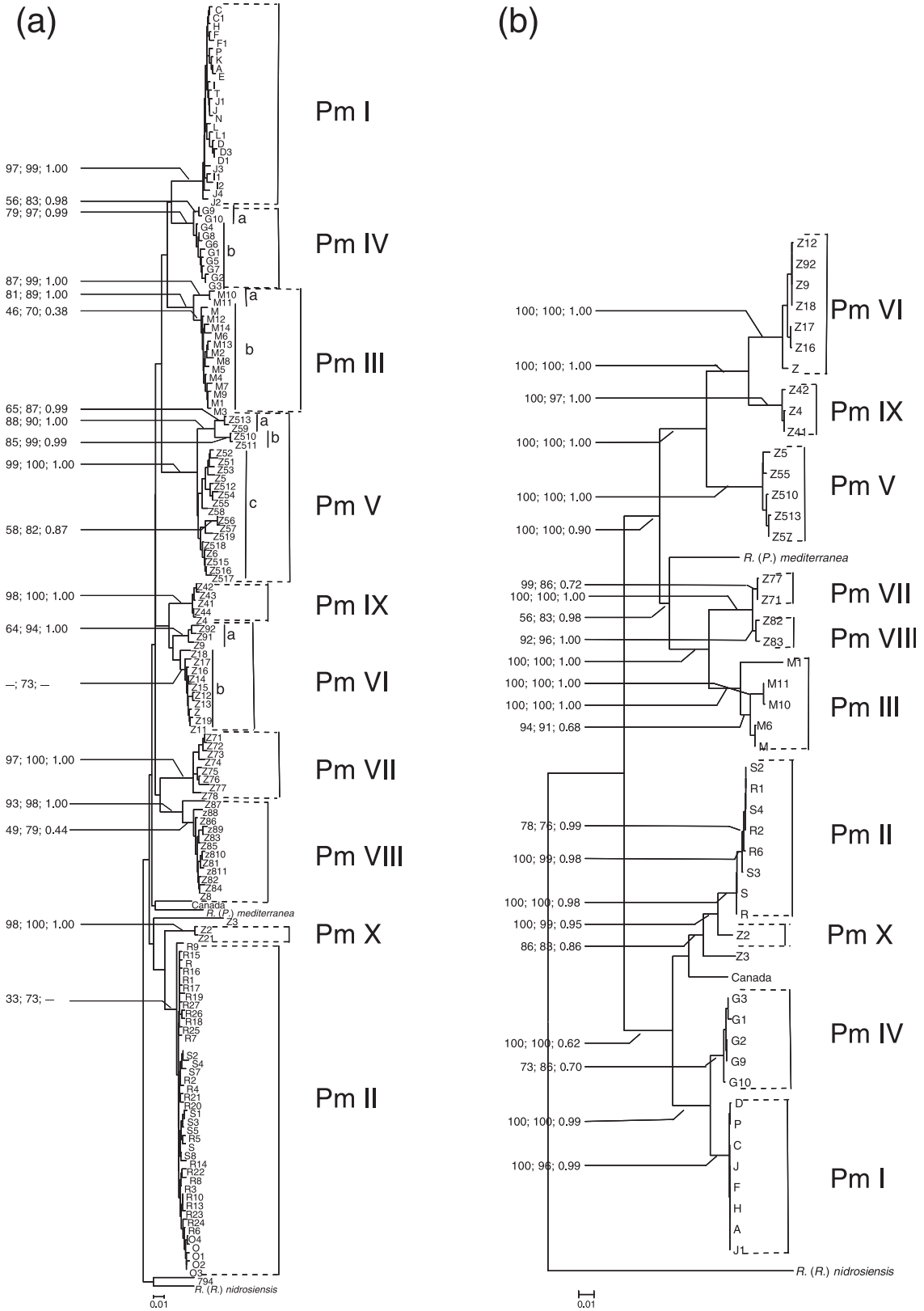

Fig. 1 *Rhabditis* (*Pellioditis*) *marina*. (a) *P*-distance neighbour-joining (NJ) tree of COI. (b) *P*-distance NJ tree of the concatenated ITS and D2D3 region. Values above branches are bootstrap values for NJ, MP and Bayesian probabilities. Cryptic species are designated by Pm (from *Pellioditis marina*), subclades are indicated by a, b or c.

Table 2 Sequence differences within and between species of the *Rhabditis* (*Pellioditis*) *marina* complex. Mean within-group sequence divergence ± standard error and maximum sequence divergence between brackets (diagonal), and mean between-group divergences ± standard error are based on *P*-distances. Sequences *Z3*, *Rhabditis* (*Pellioditis*) *mediterranea* [*R. (P.) med*] and *Rhabditis* (*Rhabditis*) *nidrosiensis* [*R. (R.) nidro*] are from Derycke *et al.* (2005, 2006)

	PmI	PmII	PmIIIb	PmIIIa	PmIV	PmV	PmVI	PmVII	PmVIII	PmIX	PmX	Z3	Ca	*R. (P.) med*
PmI	1.0 ± 0.2 (1.8)													
PmII	8.5 ± 1.3	1.0 ± 0.2 (1.8)												
PmIIIa	7.5 ± 1.3	8.5 ± 1.4	0.9 ± 0.2 (0.5)											
PmIIIb	7.9 ± 1.3	8.3 ± 1.4	2.8 ± 0.7	0.5 ± 0.3 (1.6)										
PmIV	6.3 ± 1.1	7.6 ± 1.3	7.1 ± 1.3	6.8 ± 1.3	1.0 ± 0.3 (2.3)									
PmV	9.9 ± 1.4	9.1 ± 1.3	8.4 ± 1.3	8.9 ± 1.3	8.9 ± 1.3	2.6 ± 0.5 (4.9)								
PmVI	9.8 ± 1.4	6.7 ± 1.2	7.6 ± 1.2	7.6 ± 1.2	8.5 ± 1.3	8.5 ± 1.3	2.3 ± 0.4 (5.2)							
PmVII	9.6 ± 1.4	8.1 ± 1.3	8.4 ± 1.3	8.6 ± 1.4	10.6 ± 1.5	9.8 ± 1.3	7.2 ± 1.2	1.3 ± 0.4 (2.1)						
PmVIII	9.0 ± 1.4	7.5 ± 1.3	7.1 ± 1.2	6.7 ± 1.2	8.9 ± 1.4	9.3 ± 1.3	7.8 ± 1.2	7.8 ± 1.2	1.3 ± 0.3 (4.2)					
PmIX	9.4 ± 1.5	7.1 ± 1.3	7.8 ± 1.3	6.9 ± 1.2	9.5 ± 1.5	8.3 ± 1.3	4.5 ± 0.9	7.9 ± 1.3	7.7 ± 1.3	0.5 ± 0.2 (0.8)				
PmX	10.2 ± 1.5	4.6 ± 1.0	9.1 ± 1.5	8.3 ± 1.5	8.9 ± 1.4	9.7 ± 1.4	7.6 ± 1.3	8.0 ± 1.3	8.8 ± 1.4	7.7 ± 1.4	0.5 ± 0.3 (0.5)			
Z3	11.4 ± 1.5	9.0 ± 1.4	11.2 ± 1.5	12.0 ± 1.6	11.0 ± 1.5	10.4 ± 1.5	10.1 ± 1.5	10.7 ± 1.5	11.4 ± 1.6	11.3 ± 1.5	10.6 ± 1.6	—		
Ca	9.2 ± 1.5	7.0 ± 1.2	6.7 ± 1.2	6.6 ± 1.2	8.2 ± 1.3	9.4 ± 1.4	7.7 ± 1.3	8.0 ± 1.3	8.3 ± 1.3	8.3 ± 1.4	7.7 ± 1.3	9.7 ± 1.5	—	
R. (P.) med	10.2 ± 1.5	7.7 ± 1.3	7.6 ± 1.3	8.0 ± 1.4	9.2 ± 1.4	9.7 ± 1.4	6.8 ± 1.2	9.9 ± 1.5	9.2 ± 1.4	8.8 ± 1.4	8.6 ± 1.4	11.7 ± 1.6	7.6 ± 1.4	—
R. (R.) nidro	7.3 ± 1.2	8.1 ± 1.3	7.8 ± 1.3	7.7 ± 1.3	8.5 ± 1.3	10.4 ± 1.4	9.0 ± 1.4	9.5 ± 1.4	9.3 ± 1.4	7.9 ± 1.3	9.1 ± 1.4	12.7 ± 1.7	8.1 ± 1.4	8.8 ± 1.4

Table 3 *Rhabditis* (*Pellioditis*) *marina*. AMOVA results for the species occurring in several locations and with $n > 5$. The number of individuals in the analysis (n), the amount of variation explained by differences among and within populations (percentage), the Φ statistics and the significance level (P) are shown. ***$P < 0.001$

		n	Percentage	Φ_{ST}	P
PmI		370			
	Among populations		22.29	0.22	***
	Within populations		77.71		
PmII		298			
	Among populations		36.68	0.37	***
	Within populations		63.32		
PmIII		269			
	Among populations		18.74	0.19	***
	Within populations		81.26		
PmIV		77			
	Among populations		59.72	0.60	***
	Within populations		40.28		
PmV		76			
	Among populations		39.31	0.39	***
	Within populations		60.69		
PmVI		44			
	Among populations		33.01	0.33	***
	Within populations		66.99		
PmVIII		115			
	Among populations		25.90	0.26	***
	Within populations		74.10		

(PmV, PmVI and PmVIII), of which PmV and PmVI had a very discontinuous distribution: PmV was encountered in Po, Sy (see Table 1 for sample abbreviations), in three Mediterranean and two Baltic samples, but was absent from any intermediate Atlantic locality. PmVI was rare, but has been found in the Bay of Biscay (Sa), the North Sea (Bl) and in South Africa. The remaining four species (PmIV, PmVII, PmIX, PmX) were sampled at low frequencies (Fig. 2). This, and the discontinuous distribution of PmV and PmVI, may reflect an inefficient sampling coverage rather than their real abundances and distributions.

AMOVA suggested a significant genetic structuring in all species with sufficient sample size (Table 3). Population pairwise Φ_{ST} values between PmIIIb populations on both sides of the Atlantic were generally low and nonsignificant (Appendix I, in grey). The structuring was mainly caused by the Bay of Biscay (Va, see Table 1 for sample abbreviations) and the saltwater lake location in the Netherlands (GrB). Similarly, a strong genetic differentiation was observed for PmIV between the Bay of Biscay (San) and all other locations. For PmV, the northern samples Sy and He were not significantly differentiated from each other, while the southern populations (Po, GrL, and Ay) were (Appendix I). Interestingly, the southern Po sample was not significantly differentiated from the northern Sy sample, and the Baltic sample (He) not from the Mediterranean Ay sample. Finally,

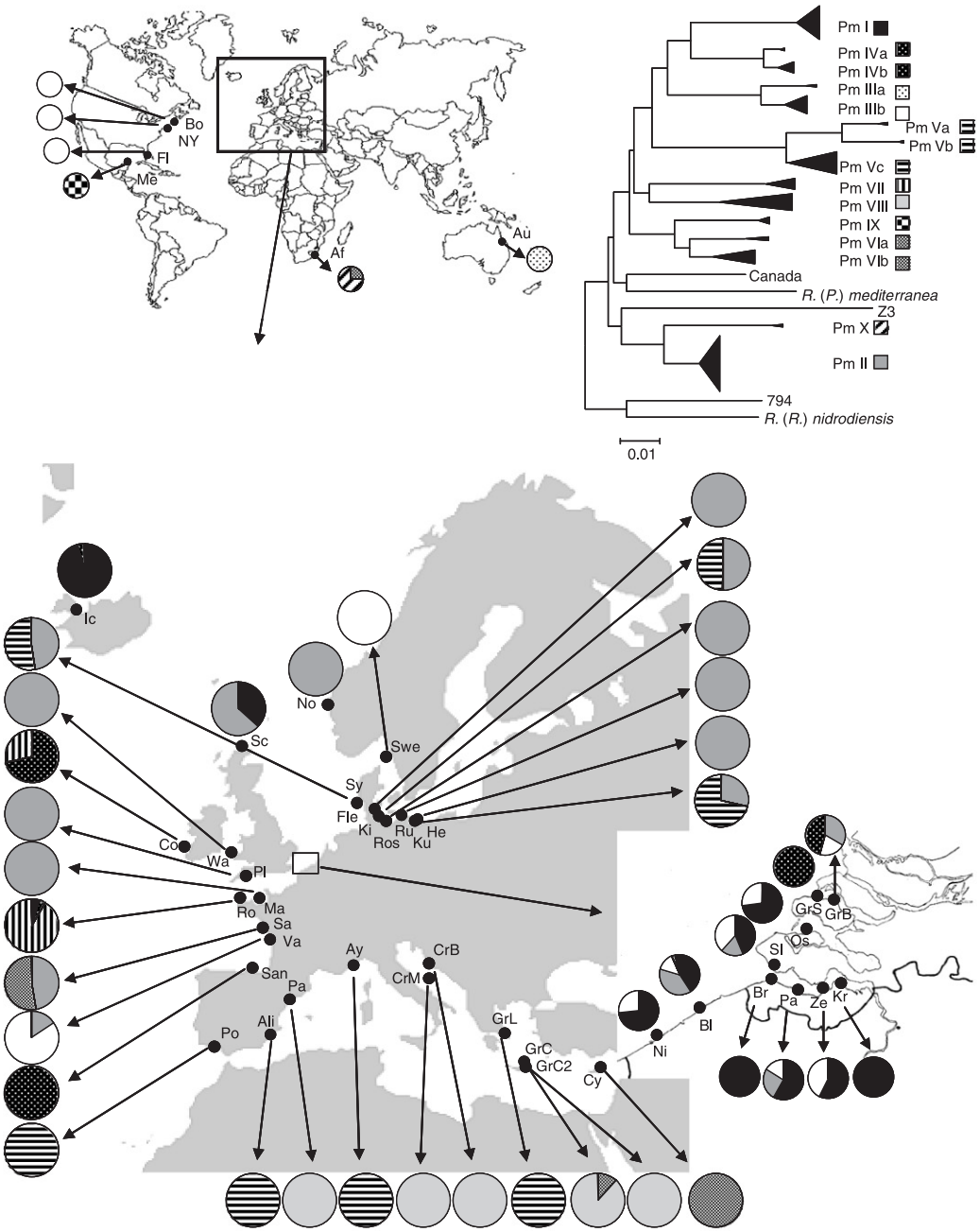

Fig. 2 *Rhabditis* (*Pellioditis*) *marina*. Distribution of the ten cryptic species in the sampled localities. PmIIIa, found in Australia, is coloured separately from PmIIIb, while other intraspecific lineages have the same colour. Abbreviations of localities are as in Table 1. Note the very low frequency of PmVI and PmX in Bl. The phylogeny of the species complex along with the colouring scheme of the pies is also shown.

all five Mediterranean populations of PmVIII were significantly differentiated from each other, even the geographically very close locations in Greece. SAMOVA indicated an additional structuring in PmI and PmII: when we imposed five groups on the PmI data, one group contained the northern locations of Scotland and Iceland, and another group contained five locations from the Southern Bight of the North Sea (Ze, Kr, Pa, Br and Sl). The haplotypes found in Sc (I3 and J4) and in Ic (I1, I2, J3 and J4) were not found in any other location. Similarly, when we imposed six groups on the PmII data, one group contained five northern locations (No, Sc, Ku, Wa, Ros) and another group contained six locations from the Southern Bight of the North Sea, English Channel and Bay of Biscay (Bl, Pa, GrB, Pl, Ma, Sa, Fig. 3). We subsequently performed a hierarchical AMOVA for PmI and PmII with two groups, one containing all

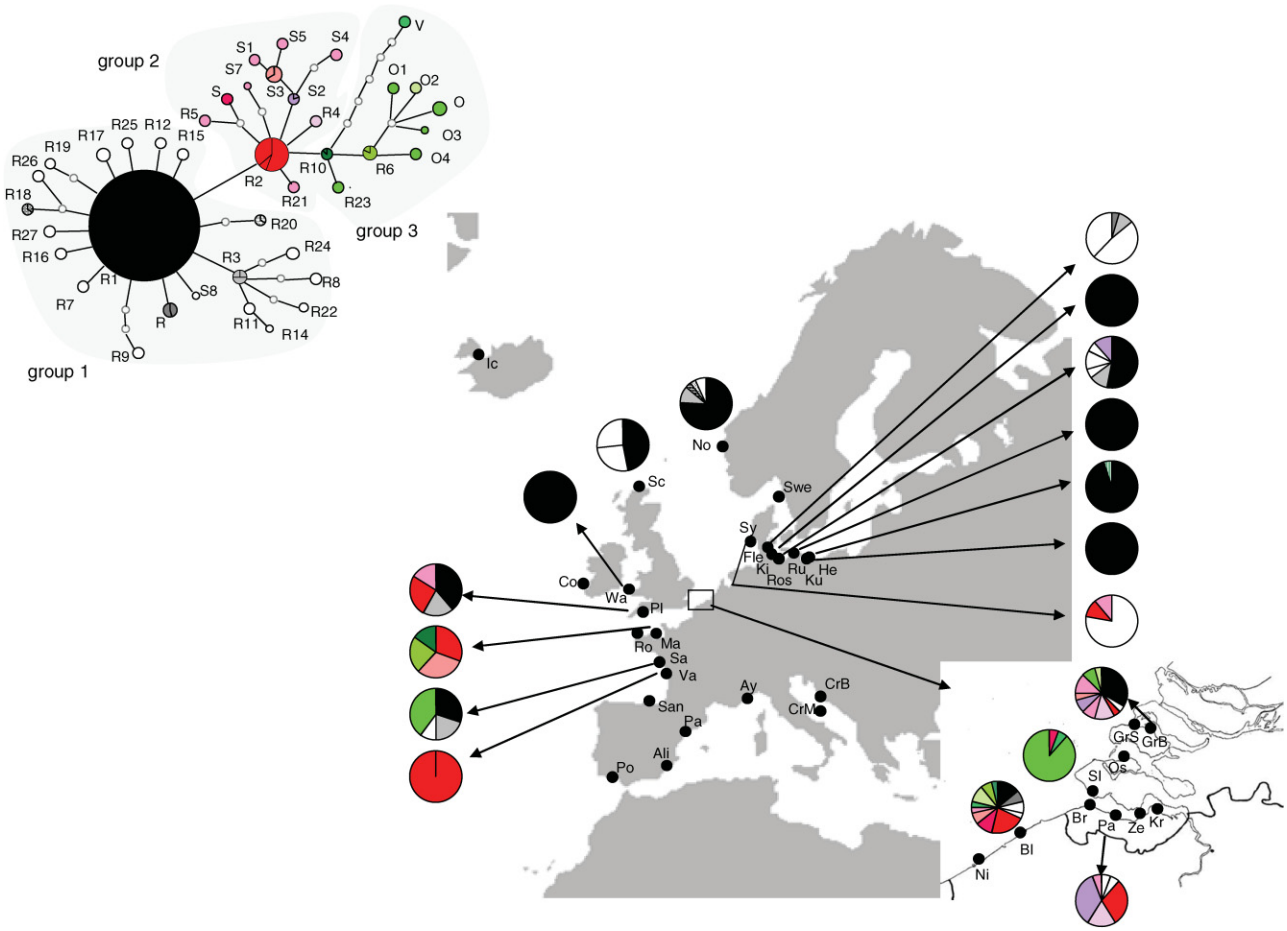

Fig. 3 *Rhabditis* (*Pellioditis*) *marina*. PmII. Distribution of haplotypes and haplotype groupings in the northeast Atlantic. Haplotypes of group 1 are coloured in black-grey-white, haplotypes of group 2 in red-pink-purple and haplotypes of group 3 in green (see panel in the left top corner, subdivision of haplotypes according to waterbasin). Haplotypes found in one location are coloured white, pink or dark green, haplotypes that are shared between locations have a shaded grey, pink or green colour.

locations above 52°N, and one containing locations situated lower than 52°N. In both species, a significant structuring could be attributed to this subdivision, suggesting a genetic break around the British Isles (Fig. 3, Table 4).

The haplotype diversity of PmII decreased slightly with latitude, but the trend was nonsignificant ($r = -0.34$, $P = 0.22$). This most likely reflects an insufficient sampling design, as rather few samples towards the latitudinal extremes were available. Haplotype diversity was highest in the Bay of Biscay and the Southern Bight of the North Sea (Appendix II), and was slightly lower in the Baltic and northern North Sea populations. Exceptions were the Fle, Ros and Sc populations, which had a similar haplotype diversity as the southern populations.

Demographic history

The maximum parsimony network yielded several unconnected haplotype groups corresponding to the different phylogenetic species (Fig. 4). Within PmV, PmVI and PmVIII, additional unconnected haplotypes/groups of haplotypes were present. Of all haplotypes, 48 were shared among sampling localities and 104 haplotypes were location specific. PmII was the most widespread species and had a continuous distribution across the northeast Atlantic (Figs 2 and 3). Its most common haplotype (R1) was also the most widespread, and was found continuously from the Bay of Biscay over the English Channel and the British Isles, into the North Sea and the Baltic (Figs 3 and 4). Many closely related haplotypes were endemic to the Baltic or to the North Sea (group 1). The second most common haplotype (R2) co-occurred with R1 in the Bay of Biscay, the English Channel, and the North Sea, but was absent from the Baltic, North Scotland and Norway. Group 2 haplotypes were restricted to the English Channel/North Sea, except for haplotype S2 which was found in low frequency ($n = 2$) in the Baltic. Of the nine haplotypes from group 3, five were endemic to the North Sea, one to the Bay of Biscay

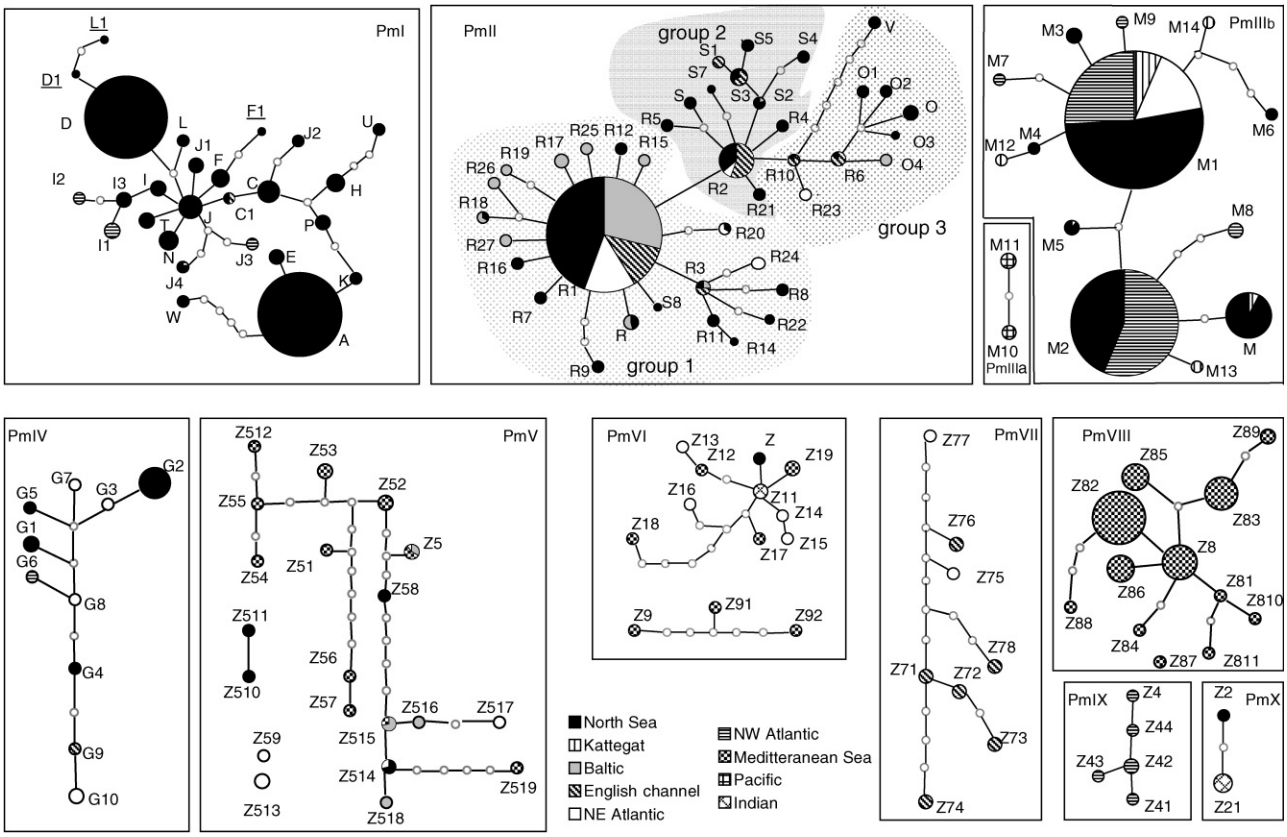

Fig. 4 *Rhabditis* (*Pellioditis*) *marina*. TCS network with 95% confidence connection between haplotypes. Circles are proportional to haplotype abundances and are shaded according to the waterbasin (see Table 1) in which they occurred. Haplotype groups are pooled in agreement with phylogenetic clades. Small empty circles are hypothetical mutations.

Table 4 *Rhabditis* (*Pellioditis*) *marina*. Hierarchical AMOVA in species PmI and PmII with groups based on SAMOVA results. The number of individuals in the analysis (*n*), the amount of variation explained by differences among and within populations (percentage), the Φ statistics and the significance level (*P*) are shown. ***$P < 0.001$

		n	Percentage	Φ_{ST}	*P*
PmI		370			
	Among groups (Ic, Sc) – (Ze, Kr, Pa, Br, Sl, Os, Bl, Ni)		43.71	0.43	***
	Among populations within groups		6.85	0.12	***
	Within populations		49.44	0.51	***
PmII		298			
	Among groups (Ru, Sy, Fle, Ku, No, Sc, Ros, Wa) – (Sl, Bl, GrB, Pa, Sa, Ma, Pl)		19.23	0.19	***
	Among populations within groups		23.12	0.29	***
	Within populations		57.65	0.42	***

and one to the Baltic. The remaining two haplotypes were shared between the English Channel and the North Sea (Fig. 3).

Tajima's *D* and Fu's *Fs* were significantly different from zero and negative only for PmII ($D = -1.6$, $P = 0.02$ and $Fs = -26.5$, $P < 0.0001$). When haplotypes of PmII were pooled according to the hierarchical AMOVA design in Table 4, the test statistics of the neutrality tests were significantly different from zero and negative for the northern group ($D = -1.6$, $P = 0.02$ and $Fs = -10.9$, $P < 0.0001$) and nearly significant for the southern group ($D = -1.21$, $P = 0.09$ and $Fs = -12.9$, $P < 0.0001$). The distribution of pairwise differences for the complete PmII data set (data not shown) and for the northern group alone fitted a model of sudden expansion (Fig. 5a). The generalized skyline plot for PmII indicated an exponential population growth starting from *c*. 145 000 years BP when applying a molecular clock of 2% per million years (myr) (Fig. 5b).

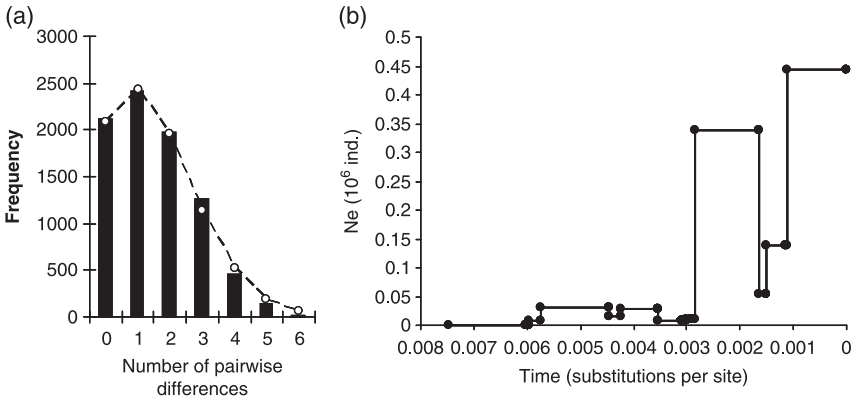

Fig. 5 *Rhabditis* (*Pellioditis*) *marina*. PmII. (a) Mismatch distribution of pairwise differences of haplotypes occurring in populations above 52°N (columns) and the expected distribution under a sudden expansion model (dashed line). (b) Generalized skyline plot for PmII mtDNA haplotypes with ε = 0.0001. The *X*-axis represents the number of substitutions since the present, the *Y*-axis represents the effective population size.

Discussion

Rhabditis (*Pellioditis*) *marina* is composed of at least 10 genetically highly divergent lineages, seven of which have previously been designated as 'cryptic' species based on molecular and morphological evidence (Derycke *et al*. 2008). Despite a substantial enlargement of the sampling area in this study, we found evidence for only three new phylogenetic species (PmV, PmVII and PmVIII) within the *R*. (*P*.) *marina* complex. These results confirm that species identification based on morphological data alone is insufficient in at least some nematode taxa, and that evolutionary methods are preferable for a straightforward delimitation of species in nematodes (Nadler 2002).

Speciation and distribution of R. (P.) marina

The phylogeographical patterns within *R*. (*P*.) *marina* are complex, and elucidating the factors responsible for speciation is therefore difficult. Allopatric speciation is considered the most common speciation mechanism in marine species (Wilke & Pfenninger 2002). At first sight, the sympatry of several cryptic species of *R*. (*P*.) *marina* challenges the allopatric speciation model, but some *R*. (*P*.) *marina* species are highly dominant in particular geographical areas (PmI, PmVII and PmVIII) and have a strong genetic structuring. All sympatric occurrences are dominated by a single species, and the most closely related species are generally allopatric (PmI and PmIV, PmVI and PmXI). Intraspecific phylogroups are also allopatrically distributed in the case of PmIII and PmIV, while this is not the case for the PmV phylogroups, which co-occur in Po and Sy, and for the PmVI phylogroups, which co-occur in Cy. The general pattern of increasing sympatry with more distantly related species is consistent with a predominant mode of allopatric speciation, and points to the occurrence of range changes after the speciation event leading to sympatry among species (Barraclough & Vogler 2000).

Alternatively, we find ecological speciation less likely in view of the distribution of PmII and PmV: both species are found under a range of quite different ecological conditions — like salinity (lowest in Baltic samples and in the Kr location in the Westerschelde estuary), temperature (lower in northern than in southern locations) and substratum (seagrass in the Mediterranean, macroalgae in all other samples) — suggesting that other factors than ecological differences have triggered speciation. Pleistocene glaciations are believed to have played a key role in the formation of new species (Avise 2000; Hewitt 2000), but it is unlikely that they initiated the speciation in *R*. (*P*.) *marina*. Values for divergence times and substitution rates for nematodes are poorly known, and the lack of any fossil record makes a good calibration of a molecular clock difficult. Nematodes have a very high metabolism due to their small body size and often have a short generation time. A molecular clock of 2% per million year (Avise 2000) will therefore most likely result in minimum divergence times. Net sequence divergences between the sister species range between 2.0–9.4%, which places the (oldest) separation event in the Pliocene somewhere between 1.0–4.7 million years ago. Speciation of the species complex may therefore have been completed before the Pleistocene. However, when speciation is seen as a gradual process rather than as a point event in time, Quaternary biogeographical factors may also have promoted the Pliocene separation events (Avise 2000).

If allopatric speciation is the primary mode of speciation, then the sympatric distribution of *R*. (*P*.) *marina* species most likely reflects recent colonization rather than a long-term stable sympatry. Epiphytic intertidal nematodes are regularly subject to suspension in the water column (Fegley 1987) and can raft on macroalgae (Thiel & Gutow 2005). Such dispersal is intrinsically random, and upon arrival ashore, population establishment is likely to be successful if conditions are favourable. In the few instances where the most closely related species co-occur, the abundance of at least one of these most closely related species is always very low (e.g. in Iceland, Roscoff). This favours the competitive exclusion hypothesis (Huisman & Wiessing 1999; Suatoni *et al*. 2006), while genetically more diverged species avoid competitive exclusion, perhaps through resource partitioning (e.g. De Mesel *et al*. 2004). Alternatively, the species present on

the algae may monopolize resources and hamper population development of newly arriving species (De Meester *et al*. 2002). Both hypotheses, competitive exclusion and monopolization of resources, imply that resources are limiting, which is difficult to assess in such highly dynamic systems.

Dispersal in free-living nematodes

As expected, a strong structuring was found in all cryptic species of sufficient sample size, corroborating our expectations based on the genetic patterns on a local scale (Derycke *et al*. 2006, 2007a). Surprisingly, no differentiation was observed between some northeast and northwest Atlantic populations of PmIIIb and between Baltic and Mediterranean populations of PmV, despite a fairly large number of specimens analysed. Furthermore, the transoceanic distribution of several species suggests that effective long-distance dispersal has occurred in at least some species of the *R.* (*P.*) *marina* complex. Genetic exchange across large oceanic distances has also been observed in other small marine eukaryotes, like foraminifers (Darling *et al*. 2000) and ciliates (Finlay *et al*. 2006). Before this study, little direct evidence for the transport of nematodes over large oceanic distances was available and the extent of passive dispersal in marine nematodes was unknown (Coomans 2002). Possible means of dispersal are rafting (we were able to isolate *R.* (*P.*) *marina* specimens from a patch of drifting algae in the North Sea), ship ballast water and migrating birds. In the case of PmIII, the presence of two haplotypes (M1, M2) at relatively high abundances on both sides of the Atlantic may be explained by multiple colonization events or by a single large dispersal propagule (Wares & Cunningham 2001). The lower *h* values in northwest Atlantic samples suggest that this dispersal has occurred from Europe towards North America, which in view of the contemporary water currents is difficult to explain. Therefore, an old dispersal event with a large propagule containing multiple haplotypes is the most plausible explanation for the trans-atlantic pattern in PmIIIb. At finer geographical scales, the high amount of shared haplotypes and the presence of only few unique haplotypes among populations in the Belgian and Dutch locations indicate that epiphytic nematodes are able to disperse at scales of several tens of kilometres (Derycke *et al*. 2007b). At intermediate geographical scales, dispersal may be restricted by water currents or local retention of nematodes in the water column (Palumbi 1994; Cowen *et al*. 2006).

Phylogeographical patterns

Phylogeographical studies have indicated that Pleistocene climate changes, and in particular the last glacial maximum (LGM), have affected the distribution of marine organisms. Four general genetic patterns have been found in a variety of marine species, and our one locus data corroborate these general patterns. First, several marine species follow an expansion model predating the LGM (see Chevolot *et al*. 2006 for a list of species, Gómez *et al*. 2007; Hoarau *et al*. 2007). The haplotype network, mismatch analysis and generalized skyline plot all indicate an expansion in PmII. Assuming that the molecular clock of 2% per million years is an appropriate mutation rate for PmII, the (oldest) timing of the biggest expansion event is *c*. 145 000 years BP and consequently also predates the LGM.

Second, three northern refugial areas in the northeast Atlantic have been proposed for marine species: the southwest coast of Ireland, the Brittany/English Channel region and the northern Iberian Peninsula (see Hoarau *et al*. 2007 for a list of species, Gómez *et al*. 2007). The *h* values in the Brittany/English Channel area are among the highest observed for PmII (Appendix II). This may be indicative of a refugial zone around Brittany/English Channel (Provan *et al*. 2005) or, alternatively, of a recolonization from several southern refugia (see Coyer *et al*. 2003). However, many interior haplotypes are distributed in the English Channel, and the genetic break around the British Isles in PmI and PmII indicated by SAMOVA, supports the hypothesis of a refugium in the English Channel (Coyer *et al*. 2003; Provan *et al*. 2005). The survival of fucoid algae during the LGM (Hoarau *et al*. 2007) may indicate that *R.* (*P.*) *marina* was also able to survive glacial periods in some areas, as it occurs predominantly on these macroalgae. We cannot, however, rule out a refugial zone located further south due to the high haplotype diversity in the Bay of Biscay sample Sa, and we have no PmII data to investigate the refugium hypotheses for the two other regions.

Third, the Southern Bight of the North Sea may be a contact zone for marine species (Garcia-Marin *et al*. 1999; Gysels *et al*. 2004; Jolly *et al*. 2005; Remerie *et al*. 2006). The high abundance of the interior R1 haplotype in Wa, Sc and No in combination with a relatively low abundance in the Southern Bight of the North Sea, suggests that group 1 was forced to expand around the British Isles into the northern North Sea, while group 2 expanded into the Southern Bight of the North Sea through the English Channel (Fig. 3). The distribution of the three PmII haplotype groups highly overlaps in the Southern Bight of the North Sea, which may be indicative of a contact zone between clades from these two separate expansion events.

Finally, a general trend of declining genetic diversity with latitude has been observed in northern temperate species as a result of founder events during expansions (Avise 2000; Hewitt 2000). Although the genetic diversity was slightly declining towards the North, *h* values were not significantly lower in the Southern Bight of the North Sea compared to the southern Bay of Biscay population. Our sampling is biased towards the North Sea, but haplotype diversity analysed by season was also high in the Southern

Bight of the North Sea (Derycke *et al*. 2006). This further supports the idea of a contact zone in the Southern Bight of the North Sea (see above). Within the Baltic, haplotype diversity is highest in the Belts Sea (Fle, Ros) and gradually decreases into the western Baltic (Appendix II). Pairwise Φ_{ST}-values between each of the northern North Sea samples (Sc, No and Sy) and the three Baltic samples (Fle, Ku and Ru) are highest when the westernmost Baltic sample (Ru) is included (Appendix I). These patterns correspond with founder events following colonization from the North Sea and have also been observed in fish (Nielsen *et al*. 2003; Nielsen *et al*. 2004; Bekkevold *et al*. 2005).

Conclusion

Contrary to our expectations, the enlargement of our study area resulted in the detection of only three new cryptic species within the *Rhabditis* (*Pellioditis*) *marina* species complex. We did find a significant structuring in all *R*. (*P*.) *marina* species, which shows that dispersal in marine nematodes living on macroalgae is limited. At the same time, we found evidence for some occasional long-distance dispersal. On smaller geographical scales (< 100 km), effective dispersal is likely to be substantial. We also expected to find a lower genetic diversity in northern areas. Although a decreasing trend with latitude was observed, this decrease was not significant. The present study further agrees well with phylogeographical patterns found in other marine species and shows the importance of range expansions and restricted gene flow as microevolutionary processes in the *R*. (*P*.) *marina* species complex. These range expansions pre-date the LGM, and the genetic diversity of PmII supports the English Channel refugium hypothesis and the idea of a contact zone in the Southern Bight of the North Sea, suggesting that the geographical changes accompanying the Pleistocene glaciations have influenced PmII. This study further illustrates the cosmopolitanism of meiofauna species. We hypothesize that the cryptic speciation in *R*. (*P*.) *marina* is the result of allopatric speciation, and explain the current sympatric distribution by random and occasional effective long-distance dispersal followed by interspecific interactions like competition and/or monopolization of resources.

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This study is part of the PhD of S. Derycke which focused on the genetic structure of marine nematode taxa. S. Derycke's postdoctoral research includes population genetics and speciation patterns of marine nematodes, and aims at developing a rapid and straightforward identification tod for marine nematodes. T. Remerie is a postdoctoral fellow interested in phylogeography and population genetics of marine invertebrates. T. Backeljau focuses on the evolution of reproductive systems and phylogeny and taxonomy of mollusks and has a broad interest in the phylogeography of marine invertebrates. A. Vierstraete and J. Vanfleteren have expertise in molecular evolution and phylogeny and J. Vanfleteren has also a long track record in biogerontology of *Caenorhabditis elegans*. M. Vincx and T. Moens are interested in taxonomy and ecology of free-living marine nematodes. T. Moens uses marine nematodes to study the relationship between biodiversity and ecosystem functioning.

Appendix I

Pairwise Φ_{ST} values for the species PmI, PmII, PmIIIb, PmIV, PmV and PmVIII. Sample abbreviations are as in Table 1. Values in bold are significantly different from zero after Bonferroni correction. Grey block highlights the Φ_{ST} values between northeast and northwest Atlantic samples

PmI	Ic	Sc	Ze	Kr	Pa	Br	Sl	Os	Bl	Ni
Ic										
Sc	0.23982									
Ze	**0.50348**	**0.43431**								
Kr	**0.54367**	**0.48309**	0.04281							
Pa	**0.45207**	**0.37200**	0.00056	0.04474						
Br	**0.56953**	**0.51714**	–0.00042	0.08115	0.03486					
Sl	**0.49403**	**0.42162**	–0.01589	0.03724	0.00073	0.00913				
Os	**0.58813**	**0.53940**	**0.16549**	0.03516	**0.13462**	**0.23841**	**0.14968**			
Bl	**0.61786**	**0.58963**	0.03150	0.13731	0.07738	–0.01077	0.04397	**0.32326**		
Ni	**0.56416**	**0.51107**	**0.19130**	**0.24561**	**0.13463**	**0.25963**	**0.16360**	**0.31391**	**0.35262**	

PmII	Ru	Sy	Fle	Ku	No	Sc	Sl	Bl	GrB	Pa	Sa	Ma	Wa	Pl	Ros
Ru															
Sy	**0.71882**														
Fle	**0.45247**	**0.53482**													
Ku	**0.56062**	**0.61072**	**0.27451**												
No	**0.59988**	**0.65544**	**0.29304**	0.07970											
Sc	**0.51069**	**0.51662**	**0.27287**	0.16617	0.14207										
Sl	**0.85833**	**0.75613**	**0.74765**	**0.80309**	**0.83853**	**0.74413**									
Bl	**0.35897**	**0.36181**	**0.30037**	**0.19390**	**0.24566**	**0.16003**	**0.49423**								
GrB	**0.35962**	0.31765	**0.29927**	0.21040	**0.25226**	0.18235	**0.54347**	0.01464							
Pa	**0.54791**	**0.47839**	**0.40294**	**0.36326**	**0.41960**	**0.35629**	**0.69194**	0.06251	0.01812						
Sa	**0.45575**	0.33374	**0.31211**	0.26581	**0.30033**	0.19984	**0.63392**	0.09935	0.11023	**0.20858**					
Ma	**0.58868**	**0.48475**	**0.50378**	**0.46454**	**0.50723**	**0.41993**	**0.55876**	0.06723	0.08868	0.11182	0.25298				
Wa	**0.88763**	**0.69415**	0.25214	0.07234	–0.01785	0.17278	**0.85884**	0.19758	0.19811	**0.39672**	0.29425	**0.48946**			
Pl	**0.42050**	**0.43740**	**0.25683**	0.15641	**0.16545**	0.13412	**0.67432**	0.04346	0.04561	0.09716	0.12487	**0.20789**	0.14590		
Ros	**0.44907**	**0.48782**	**0.22523**	0.06339	0.02174	0.10013	**0.75917**	**0.14484**	0.13715	0.24844	0.16481	**0.38378**	0.01622	0.06947	

PmIII	Ze	Pa	Sl	Os	Bl	Ni	GrB	Va	Bo	NY	Fl	Swe
Ze												
Pa	0.14602											
Sl	0.00293	0.18039										
Os	0.10564	0.10990	0.13707									
Bl	–0.00236	0.12495	0.01240	0.01949								
Ni	0.07821	**0.28362**	0.16000	0.20690	0.09091							
GrB	**0.26118**	**0.48590**	**0.23589**	**0.51924**	0.36940	**0.54270**						
Va	**0.24185**	**0.59511**	0.21105	**0.62352**	**0.41105**	**0.70365**	**0.40370**					
Bo	**0.16431**	**0.28240**	**0.23820**	0.21338	**0.16071**	0.04638	**0.50341**	**0.54742**				
NY	0.03714	**0.30790**	0.06856	**0.27870**	0.09690	0.10593	**0.34582**	**0.32801**	**0.19282**			
Fl	0.02463	**0.32330**	0.06547	**0.28160**	0.08305	0.07403	**0.39934**	0.41207	0.17279	–0.00672		
Swe	–0.01756	0.15604	–0.03649	0.12971	0.00342	0.14741	0.24956	**0.25454**	**0.21279**	0.04986	0.04935	

PmIV	GrS	Co	San	GrB
GrS				
Co	0.12162			
San	**0.79161**	**0.91605**		
GrB	0.01688	0.24213	**0.86344**	

Appendix I *Continued*

PmV	Sy	He	GrL	Ay	Po
Sy					
He	0.27030				
GrL	**0.43428**	**0.47821**			
Ay	**0.24862**	0.24861	**0.34461**		
Po	0.24970	**0.42042**	**0.47565**	**0.41130**	

PmVI	Bl	Af	Sa	Cy
Bl				
Af	**1.00000**			
Sa	**0.46406**	0.29028		
Cy	0.28860	0.25958	**0.33124**	

PmVIII	CrB	CrM	GrC	Pal	GrC2
CrB					
CrM	**0.30059**				
GrC	**0.32891**	**0.17376**			
Pal	**0.33161**	**0.18798**	**0.28619**		
GrC2	**0.21527**	**0.23362**	**0.22946**	**0.29951**	

Appendix II

Rhabditis (*Pellioditis*) *marina*. PmII. Haplotype diversity (*h*) of PmII across latitude. Error bars are standard deviations. Geographical regions are indicated, sample abbreviations are as in Table 1