Metazoan parasites of freshwater cyprinid fish (*Leuciscus cephalus*): testing biogeographical hypotheses of species diversity

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SUMMARY

The diversity and similarity of parasite communities is a result of many determinants widely considered in parasite ecology. In this study, the metazoan parasite communities of 15 chub populations (*Leuciscus cephalus*) were sampled across a wide geographical range. Three hypotheses of biogeographical gradients in species diversity were tested: (1) latitudinal gradient, (2) a 'favourable centre' *versus* 'local oasis' model, and (3) decay of similarity with distance. We found that the localities in marginal zones of chub distribution showed lower parasite species richness and diversity. A latitudinal gradient, with increasing abundance of larvae of *Diplostomum* species, was observed. There was a general trend for a negative relationship between relative prevalence or abundance and the distance from the locality with maximum prevalence or abundance for the majority of parasite species. However, statistical support for a 'favourable centre' model was found only for total abundance of Monogenea and for larvae of *Diplostomum* species. The phylogenetic relatedness of host populations inferred an important role when the 'favourable centre' model was tested. Testing of the hypothesis of 'decay of similarity with geographical distance' showed that phylogenetic distance was more important as a determinant of similarity in parasite communities than geographical distance between host populations.

Key words: parasite diversity, community similarity, biogeography, phylogeny, distance decay, favourable centre.

INTRODUCTION

The role of parasites in nature is important. Parasites and their hosts represent a common subject of many recent biological studies conducted specifically to elucidate the ecological and biogeographical patterns of parasite diversity and the determinants of species richness in parasite communities. Factors influencing parasite communities are numerous. According to Poulin and Rohde (1997), the present-day composition and biological diversity of parasite communities are the result of losses and acquisitions of parasite species during the evolutionary history of their hosts. Environmental factors, host ecological traits, such as diet and body size as well as geographical range are also important determinants of parasite communities (Poulin, 1997). Recently, several ecological studies have emphasized the role of geographical distance between host populations in determining the similarity of parasite assemblages (Kennedy and Bush, 1994; Poulin and Morand, 1999; Poulin, 2003, 2007; Fellis and Esch, 2005; Oliva and González, 2005).

Three hypotheses are presently applicable to the analysis of biogeographical gradients of parasite biodiversity: (1) latitudinal gradient, (2) a 'favourable centre' *versus* 'local oasis' model, and (3) distance decay, i.e. the role of geographical distance between host populations in the structure (similarity and disimilarity) of parasite communities.

Following general ecological theory regarding increased biodiversity in the tropics, the analysis of parasite assemblages of fish in tropical regions has led to the suggestion that parasite communities exhibit greater diversity in tropical latitudes due to higher evolutionary rates (Rohde, 1992). Latitude is considered a major biogeographical factor that can influence parasite diversity (Rohde, 1992; Poulin and Rohde, 1997). Several climatic factors are presumed to show a consistent pattern with this latitudinal gradient of parasite biodiversity (Rohde, 1992), particularly as temperature changes may cause peaks in parasite abundance to shift northward or southward (Poulin, 2007). Two alternative hypotheses, namely the 'time' hypothesis (diversification of communities in time: host age and endemism could provide an explanation for the high diversity of parasite communities) and the 'latitudinal' hypothesis,

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were suggested by Kennedy (1995) to explain the differences in composition of parasite communities.

Differences in parasite biodiversity across the geographical range of the host may be explained by the 'favourable centre' hypothesis based on the assumption that species' abundance is greatest at the centre of the geographical range of the host, and declines toward the margins. Abundance may be coupled to environmental gradients (Sagarin and Gaines, 2002), a species reaching its highest abundance at the centre of its range, where optimal conditions for survival and reproduction exist. With increasing distance from this optimal site the conditions become less favourable and population size declines. This 'favourable centre' model predicts the unimodal distribution of species abundance in space (Poulin and Dick, 2007). The multimodal distribution of species abundance across its geographical range is predicted by the 'local oasis' model (Poulin and Dick, 2007) due to changing environmental conditions over space or time.

The third, 'distance decay' hypothesis (decay of similarity with distance), results from the general prediction that biological similarity decreases with increased geographical distance. 'Decay of similarity with geographical distance' in ecological communities is explained by several different types of mechanism (Poulin and Morand, 1999; Oliva and González, 2005). According to Nekola and White (1999), the increase of distance is associated with the decay of environmental similarity. Another frequently mentioned cause of 'distance decay' is spatial configuration and landscape structure, features that limit the dispersal rate of organisms (Garcillán and Ezcurra, 2003; Soininen *et al*. 2007) i.e., geographical barriers and different dispersal abilities of organisms are responsible for the decay of similarity. Finally, the decay in community similarity may be the result of ecological drift, random dispersal and random speciation, according to a neutral theory of biodiversity and biogeography, rather than by environmental heterogeneity (Hubbell, 2001).

Parasite communities in host species with close phylogenetic relationships between populations across their distribution range are presumed to be a suitable model for investigating the hypothesis of the decay of similarity with geographical distance. Presently, there is considerable evidence that the distance between host populations affects similarity in the composition of parasite communities (Poulin and Morand, 1999; Poulin, 2003; Oliva and González, 2005). However, the information to confirm the close phylogenetic relationships between host populations was not evaluated in those studies. González and Moreno (2005) suggested that fish ectoparasites were more appropriate than endoparasites for the analysis of decay rates with geographical distance, because of their direct life cycles and high host specificity. Nevertheless, the local abundance of parasite species does not depend only on the local availability of intermediate hosts (in the case of endoparasites), but also on the local availability of fish hosts and abiotic conditions (Poulin and Dick, 2007).

We selected the chub (*Leuciscus cephalus*), a common European cyprinid species, to investigate the similarity of metazoan parasite communities. Chub is a highly abundant and widespread species in Europe. Its natural geographical distribution extends from the Ural basin to the northern part of Spain, and from the south of Sweden to Italy (Berg, 1949). It is considered a Danubian species (Banarescu, 1989, 1990). Several endemic subspecies belonging to the *L. cephalus* complex (Bianco, 1983) have been described in the Mediterranean region (Durand *et al*. 2000), whereas central European chub populations have been recognized as one subspecies (Banarescu, 1991). The natural genetic variability of chub and its biological features are not influenced by introduction of fish (Guinand *et al*. 1996). Therefore, this species represents a suitable model for studying dispersion, evolutionary processes and phylogeography. Durand *et al*. (2000) investigated the divergence in cytochrome *b* gene sequences among and within chub populations in European rivers and identified 4 highly divergent mitochondrial lineages (or haplotypes), implying that there are 4 geographical lineages: Western, Eastern, Adriatic and Aegean.

The present study focused on the analysis of metazoan parasite communities from 15 chub populations from European rivers. (1) The latitudinal and associated temperature gradient hypothesis, (2) the 'favourable centre' *versus* 'local oasis' model, and (3) the 'decay of similarity with geographical distance' hypothesis were tested. As present-day parasite diversity is the consequence of current and historical ecological processes (Ricklefs, 1987), phylogenetic distances (based on mitochondrial cytochrome *b* gene sequence data) among chub populations were included in the analyses.

MATERIALS AND METHODS

Fish sampling and study area

A total of 311 individuals from 15 populations of chub from 7 different countries were collected from European rivers across much of the geographical distribution of this fish (Fig. 1). Detailed information on the localities (latitude, water temperature, sample size and host body size and weight) is shown in Table 1. All fish were sampled in the summer (including June, July and August) from 2004 to 2006. Summer periods were chosen to minimize potential seasonal differences in parasite communities among samples. Because host body size and sample size represent key determinants of parasite species richness, we sought to eliminate the influence of these

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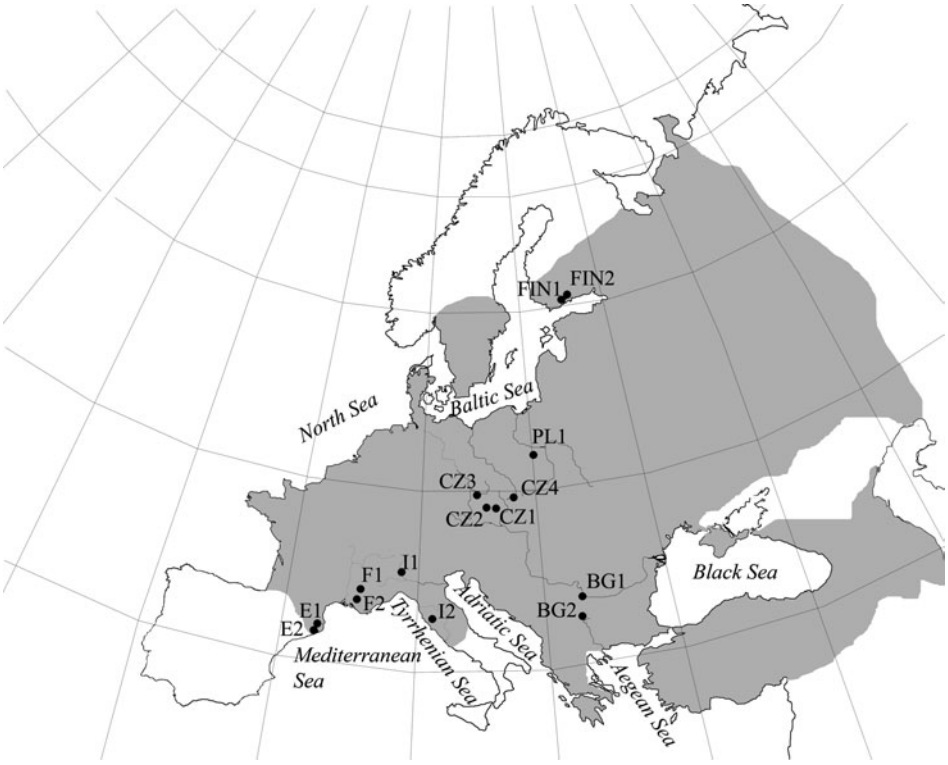

Fig. 1. Map of our sampling sites encompassing a wide range of the geographical distribution of chub in Europe modified from Durand *et al*. (2000).

factors by using a similar sample size for each locality and selecting individuals of approximately equal body size. Fish were caught using electro-fishing and transported live to the laboratory in barrels with the original oxygenated water. All fish were killed in the laboratory by severing the spinal cord. Total length (in cm) and body weight (in grams) were recorded. The fish were dissected and examined for all metazoan parasites; the external organs (skin, fins and gills) were examined for ectoparasites (Monogenea, Crustacea, Mollusca and Hirudinea), the eyes and all the viscera were examined for endoparasites (Trematoda, Cestoda, Acanthocephala and Nematoda), following the method of Ergens and Lom (1970). Most of the parasites were removed, fixed according to standard methods and determined to the species level using a light microscope (Olympus BX50) equipped with phase-contrast, differential interference contrast (DIC) and Digital Image Analysis (Olympus MicroImage™ for Windows 95/98/NT 4.0 (Olympus Optical Co.)).

Data analysis

Prevalence (percentage of infected fish in each sample) and mean intensity of infection (mean number of parasites per infected host) were calculated according to Bush *et al*. (1997) for each locality sampled. The number of parasite individuals within a considered host sample, i.e., within one locality was referred to as abundance. The structure of metazoan parasite communities was analysed at the infracommunity (all parasites in a single host) and component community (all parasites in a host population) levels (Margolis *et al*. 1982; Kennedy, 1995; Bush *et al*. 1997). Parasite diversity was measured in each chub population using several indices according to Magurran (1988): the Brillouin diversity index for each parasite infracommunity, the Shannon diversity index and parasite species richness for each parasite component community.

Similarity in parasite communities between fish populations was determined using the qualitative Jaccard index on the presence/absence matrix or the quantitative Morisita index on abundance data. The Jaccard index ranges from zero (no parasite species is common between two host populations) to one (two host populations share exactly the same parasite species). The Morisita index is sensitive to species proportions and not to absolute abundances and has been found to be insensitive to species richness and sample size (Magurran, 1988). Similarity indices were computed using PAST (PAlaeontologicalSTatistics, v.1.77, http://folk.uio.no/ohammer/past/). Also, geographical distance (metric) between pairs of fish populations was calculated from a map (www.gmap-pedometer.com).

DNA sequencing of the cytochrome b *gene from chub*

Genomic DNA was extracted (DneasyTM Tissue Kit, Qiagen) from an ethanol-preserved fish fin. An

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Table 1. Characteristics of the localities studied, their abbreviations and data on latitude, water temperature, sample size for each locality, and total length and weight of fish investigated

(CZ, Czech Republic; F, France; I, Italy; BG, Bulgaria; FIN, Finland; E, Spain, PL, Poland.)

The locality	The coordinates	Water temperature (°C)	Sample size (*n*)	Total length (cm)		Body weight (g)	
				Mean	±S.D.	Mean	±S.D.
Mustijoki – FIN2	60·43°N 25·39°E	22	21	24·88	±3·08	162·64	±68·91
Keravanjoki – FIN1	60·35°N 24·84°E	21	21	27·76	±4·08	277·15	±111·15
Mroga – PL1	52·01°N 19·39°E	18·5	21	27·67	±3·03	225·66	±73·67
Labe – CZ3	50·15°N 15·81°E	18	18	16·39	±4·28	48·98	±47·35
Odra – CZ4	49·68°N 18·01°E	13	20	22·23	±2·96	95·33	±40·98
Jihlava – CZ2	49·21°N 15·92°E	11·3	20	20	±1·29	75·28	±12·73
Svitava – CZ1	49·14°N 16·62°E	15·4	19	27·12	±3·17	228·04	±77·08
Ticino – I1	45·42°N 8·83°E	19	22	24·36	±3·48	138·15	±58·49
Le Buech – F1	44·21°N 5·93°E	16	20	20·76	±3·04	82·51	±34·28
Vidbol – BG1	43·93°N 22·83°E	19·5	21	23·9	±2·40	146·57	±53·25
L'Arc – F2	43·51°N 5·49°E	22	19	22·02	±3·72	104·8	±75·17
Merse – I2	43·14°N 11·29°E	25	22	22·23	±2·43	92·55	±33·42
Struma – BG2	42·17°N 23·06°E	24	23	19·41	±1·86	124·8	±46·69
Ser River – E1	42·17°N 2·73°E	21·3	23	22·06	±3·09	112·26	±66·56
Santa Coloma – E2	41·85°N 2·66°E	20·7	21	18·68	±1·54	59·9	±14·43

aliquot of 30 ng of genomic DNA was amplified using the Mastercycler ep gradient S (Eppendorf) in 30 μl of PCR reaction buffer with 1·5 mM $MgCl_2$, 200 μM dNTPs, 0·75 unit of *Taq* DNA Polymerase (Fermentas) and 0·5 μM of each primer – LACB (5′-GTGACTTGAAAAACCACCGTTG-3′; modified from Schmidt and Gold, 1993) and HBCB (5′-CCTCGTTGTTTTGAGGTGTGTA-3′; Dowling *et al*. 2002). The reactions were amplified for 35 cycles of 94 °C for 1 min, 53 °C for 1 min and 72 °C for 2 min; then an initial denaturation at 94 °C for 2 min and a final extension at 72 °C for 10 min. PCR products were checked on 1·5% agarose gel and were then purified by the Wizard® SV Gel and PCR Clean-Up System (PROMEGA) and sent for sequencing to Macrogen Inc. (Korea). Given that haplotype and nucleotide diversities for cytochrome *b* sequences are very low within chub populations (Durand *et al*. 2000), 5 individuals per chub population were sequenced. For phylogenetic analyses, DNA sequences were examined using Sequencher software (Gene Codes Corp.) and aligned in BioEdit v.7.0.5.3 (Hall, 1999) using Clustal W multiple alignment.

Distance trees were generated with a neighbour-joining (NJ) algorithm, based on the parameters selected by ModelTest (Posada and Crandall, 1998). Maximum likelihood (ML) analysis was conducted on the most appropriate model determined by ModelTest using hierarchical likelihood ratio tests. The search for the best ML tree was done using a branch-swapping algorithm (TBR, tree bisection reconnection). A maximum parsimony (MP) analysis was performed using an heuristic search, with a stepwise random addition sequence running on unweighted informative characters. Support values for internal nodes were estimated using a bootstrap resampling procedure after 1000 replicates (Felsenstein, 1985). The TBR for MP and NNI (nearest neighbour interchange) branch-swapping algorithms for ML analysis were used. All analyses were performed with PAUP*4b10 (Swofford, 2002). Bayesian inference analysis (BI) was conducted using MrBayes 3.1 program (Ronquist and Huelsenbeck, 2003); this analysis was performed on the most appropriate model determined by ModelTest using hierarchical likelihood ratio tests. Four Monte Carlo Markov chains (MCMC) were run for 200 000 generations, with trees being sampled every 10 generations. Log likelihoods of the saved trees were viewed graphically, and all trees before stationary were discarded as 'burn-in'. Two replicates were conducted for the Bayesian runs. The posterior probabilities (PP) of the phylogeny and its branches were determined for all trees left in the plateau phase with the best ML scores. The trees obtained by different phylogenetic methods were compared by the Shimodaira-Hasegawa (SH) test implemented in PAUP*4b10. No statistical differences ($P>0·05$) were detected, such that the phylogenetic tree obtained from the BI analysis is presented. The different haplotypes were deposited in GenBank under the Accession numbers EU791864 to EU791885.

Statistics

Spearman's rank correlation coefficient was used to assess the relationships between sample size or body size and parasites among populations (using parasite species richness or abundance). This correlation coefficient was also applied to test the hypothesis of climate (i.e., effect of water temperature in this case)

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and latitudinal effects. When the correlation coefficients were calculated between abundance of 11 parasite species and both water temperature and latitude, a meta-analysis of all independent tests was performed to obtain a general trend. The Kruskal-Wallis non-parametric ANOVA with multiple comparisons of mean ranks was used to test differences in infracommunity and component community species richness between localities.

The hypothesis of 'favourable centre' *versus* 'local oasis' model was tested following Poulin and Dick (2007). First, abundance data for parasite groups were analysed to test the prediction of the 'favourable centre abundance' model. Data on abundance of parasite groups (i.e., the proportion of abundance in each locality to the maximal values of abundance) were arcsin-transformed prior to parametric tests. Prevalence was not analysed for parasite groups, because the maximal value of prevalence, i.e., 100%, was recorded in more than one locality for several groups. Subsequently, abundance and prevalence data of the 11 parasite species with the highest values of these parameters were analysed separately. Data were log-transformed because the distribution of data fit the assumptions of normality (tested by Lilliefors test for normality). The locality with maximal values of abundance or prevalence was used as a reference and not included in the analyses to prevent an artificially generated negative relationship. Zero values (i.e., absence of parasites) were also excluded from the analyses, following Poulin and Dick (2007). The geographical distance between each locality and the reference locality was measured. The phylogenetic distance between each locality and the reference locality was calculated in MEGA 3.1 (Kumar *et al*. 2004). These distances were transformed in $\sqrt[3]{}$ to achieve a normal distribution, following Legendre and Legendre (1998). Correlation coefficients were computed between the relative values of abundance or prevalence and both geographical and phylogenetic distances. Subsequently, partial correlation coefficients between abundance or prevalence and geographical distance, after eliminating the effect of phylogenetic distance, were calculated. All correlation analyses were performed using the software STATISTICA v. 8.0 for Windows. The meta-analytical technique was used to extract a general correlation between abundance and two distances using the software Comprehensive Meta-Analysis (www.Meta-Analysis.com).

Multiple regression analysis with 'backward elimination' on distance matrices was used, following Legendre *et al*. (1994). The coefficient of determination of multiple regression and the partial regression coefficients were tested for significance using permutation methods considered appropriate for each type of dependent-matrix variable. This method is useful for testing correlations between variables representing the values between species pairs, i.e., quantitative and qualitative similarity in parasite communities, and geographical and phylogenetic distances in this study. Other raw variables, i.e., water temperature and latitude, were transformed into distance matrices by computing Euclidean distances between raw values. Probabilities were computed after 999 random permutations of the dependent matrix. Calculations were performed in Permute 3.4 for Macintosh (written by P. Casgrain, available on http://www.bio.umontreal.ca/casgrain/en/labo/permute/index.html).

RESULTS

Parasite fauna of chub

Characteristics of the 15 localities studied in 7 different countries and of the fish collected in each locality are shown in Table 1. Metazoan parasites belonging to 8 higher taxa (groups) were found representing 16 species of Monogenea, 5 species of Crustacea, 2 species of Hirudinea, 15 species of Trematoda, 3 species of Acanthocephala, 11 species of Nematoda, 9 species of Cestoda, and glochidium spp. (larval stages of Mollusca) (Table 2). Three species of ectoparasites occurred most frequently and were distributed throughout the study area except for several marginally situated localities; *Gyrodactylus vimbi* (except locality PL1), *Dactylogyrus vistulae* (except localities FIN1 and PL1) and *D. folkmanovae* (except E1 and PL1). Metacercariae of the trematode *Diplostomum* sp. and the acanthocephalan *Pomphorhynchus laevis* were the most frequently observed endoparasites. A maximum of 22 parasite species were recorded in a single locality. Chub collected from localities in the northern parts of the study area mainly harboured endoparasites, whereas those collected from central and southern localities harboured more ectoparasites (especially Monogenea) with the exception of locality F2 in which Cestoda represented the dominant parasite group (Fig. 2).

Prevalence and mean intensity were computed for each parasite species from each locality (Table 2). No parasites were found in only 2 of 311 individuals. The highest values of prevalence and intensity of infection occurred for 4 monogenean species, *Dactylogyrus folkmanovae, D. vistulae*, *D. prostae* and *Gyrodactylus vimbi*. The prevalence of 2 crustacean species was high in 2 Bulgarian, 1 Italian and 1 Spanish population, although the intensity of infection was low. The prevalence and intensity of infection of endoparasites were generally low, with several exceptions, which may represent a habitat effect. There was a high prevalence and intensity of infection of *Diplostomum* sp. in northern localities (Finland and Poland). The acanthocephalan endoparasite, *P. laevis*, was not found in fish from northern and most southern localities.

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Table 2. Metazoan parasite species found in the chub populations from European Rivers

(The data on the localization within host, prevalence (percentage of infected hosts, first line), mean intensity (second line) and range of the intensity of infection (third line) for each parasite species are included. The species marked by asterisks were found only in a single locality.)

Parasite species/ Population	Micro-habitat	FIN2	FIN1	PL1	CZ3	CZ4	CZ2	CZ1	I1	F1	BG1	F2	I2	BG2	E1	E2
Monogenea																
Gyrodactylus vimbi		28·6	4·8		88·8	15·0	100	36·8	9·1	25·0	57·1	15·8	9·1	82·6	30·4	76·2
Schulman,	F, S	1·67	1	—	13·75	1·33	59·35	2·86	1	3	2·25	1·33	1·75	39·6	3	2·62
1953		(1–5)	(1)		(3–44)	(1–2)	(1–270)	(1–10)	(1)	(1–6)	(1–6)	(1–2)	(1–3)	(6–149)	(1–8)	(1–6)
Gyrodactylus lomi					72·2	20·2		5·2					22·7		8·7	
Ergens et Gelnar,	F	—	—	—	3·7	1	—	1	—	—	—	—	2	—	2	—
1988					(1–14)	(1)		(1)					(1–5)		(1–3)	
Gyrodactylus prostae					5·6		15·0		22·7					17·4		9·5
Ergens,	F, S, G	—	—	—	1	—	2·70	—	5·6	—	—	—	—	2·5	—	1·5
1963					(1)		(1–6)		(1–22)					(1–4)		(1–2)
Gyrodactylus					11·1	5·0				35·0				39·1		14·3
gasterostei Gläser,	G, F, S	—	—	—	1·5	1	—	—	—	3	—	—	—	3·9	—	2
1974					(1–2)	(1)				(1–6)				(1–22)		(1–3)
Gyrodactylus gracili-							30·0	10·5	4·5	15·0						
hamatus Malmberg,	F, S	—	—	—	—	—	4·00	1	1	1	—	—	—	—	—	—
1964							(1–8)	(1)	(1)	(1)						
Gyrodactylus																
*kathariner*i										5·0						
Malmberg,	G, F, S	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—
1964*										(2)						
Dactylogyrus vistulae		23·8			88·8	50·0	60·0	84·2	86·4	20·0	80·9	63·2	18·2	100	73·9	85·7
Prost,	G	1·6	—	—	8·13	3·3	6·16	29·06	12·21	2·5	7·82	10·69	1·75	22·9	8·82	4·61
1957		(1–3)			(1–34)	(2–6)	(1–22)	(2–149)	(1–34)	(1–4)	(1–47)	(1–55)	(1–4)	(1–72)	(1–25)	(1–15)
Dactylogyrus fallax					50·0		85·0	10·5								
Wagener,	G	—	—	—	1·67	—	3·17	3	—	—	—	—	—	—	—	—
1857					(1–4)		(1–9)	(2–4)								
Dactylogyrus folkma-		66·7	61·9		77·8	90·0	60·0	89·5	63·6	85·0	95·2	42·1	45·5	95·7		4·8
novae Ergens,	G	3·43	3·61	—	6·29	40·83	14·25	15·65	1·43	3·59	30·8	2·38	2·3	16·22	—	1
1956		(1–7)	(1–17)		(1–23)	(6–100)	(1–38)	(1–47)	(1–5)	(1–8)	(1–63)	(1–5)	(1–6)	(2–50)		(1)
Dactylogyrus prostae		52·4	28·6		66·7	85·0	35·0		77·3	65·0	100		77·3			95·2
Molnár,	G	1·64	1·83	—	4·92	7·24	1·57	—	5·41	5	48·2	—	4·12	—	—	29·6
1964		(1–3)	(1–4)		(1–21)	(1–15)	(1–3)		(1–23)	(1–16)	(1–204)		(1–11)			(4–81)
Dactylogyrus					72·2	80·0	40·0			35·0	71·4					57·1
nanoides Gussev,	G	—	—	—	1·61	3·87	3·5	—	—	3·71	3·27	—	—	—	—	1·33
1966					(1–4)	(1–22)	(1–8)			(1–7)	(1–9)					(1–3)
Dactylogyrus vrano-					22·2	65·0	10·0			20·0	90·5		13·6	13·0		
viensis Ergens,	G	—	—	—	1·25	2·69	5·0	—	—	1·75	5·52	—	1·33	1·67	—	—
1956					(1–2)	(1–8)	(1–9)			(1–2)	(1–22)		(1–2)	(1–2)		

Dactylogyrus naviculoides Ergens, 1956*	G	—	—	—	—	—	—	—	90·9 6 (1–17)	—	—	—	—	—	—	—
Dactylogyrus spp.	G	—	—	—	72·2 3·5 (1–16)	80·0 7·87 (1–24)	40·0 4·5 (1–11)	31·5 3·16 (1–6)	22·7 2 (1–3)	30·0 1·5 (1–2)	90·5 10·42 (1–38)	—	—	30·4 2·86 (1–8)	—	—
Paradiplozoon megan (Bychowsky et Nagibina, 1959)	G	—	—	—	5·6 1 (1)	40·0 1·5 (1–3)	—	78·9 3·67 (1–6)	77·2 4·41 (1–12)	—	—	15·7 2 (2)	40·9 2·55 (1–10)	—	13·1 1 (1)	4·8 1 (1)
Paradiplozoon homoion (Bychowsky et Nagibina, 1959)	G	—	—	9·5 1 (1)	11·1 1·5 (1–2)	—	—	—	—	—	—	—	—	17·4 1 (1)	21·7 2 (1–4)	33·3 2·28 (1–5)
Crustacea																
Lamproglena pulchella Nordmann, 1832	G	—	—	—	—	5·0 1 (1)	—	—	27·3 1·33 (1–2)	20·0 2 (1–2)	76·2 1·93 (1–7)	—	72·7 1·87 (1–5)	56·5 3·31 (1–9)	—	—
Ergasilus sieboldi Nordmann, 1832*	G	—	—	—	5·6 1 (1)	—	—	—	—	—	—	—	—	—	—	—
Argulus coregoni Thorell, 1864	S	19·1 1·25 (1–2)	4·8 1 (1)	—	—	—	—	—	4·5 1 (1)	—	—	—	4·5 1 (1)	—	—	—
Argulus foliaceus (Linnaeus, 1758)*	S	—	—	—	—	—	—	—	—	—	—	—	—	—	8·7 2 (1–3)	—
Lernaea sp. juv.*	S	—	—	—	—	—	—	—	—	—	—	—	—	—	69·5 1·5 (1–3)	—
Hirudinea																
Piscicola geometra (Linnaeus, 1761)	BS	4·8 1 (1)	—	—	—	—	—	15·8 2·33 (1–4)	—	—	—	—	—	—	—	—
Codonobdella truncata Grube, 1873*	BS	—	—	—	—	—	—	—	—	—	—	—	4·5 1 (1)	—	—	—
Mollusca																
glochidium spp. (larv.)	G, F	38·1 13·63 (2–37)	57·1 9·91 (1–36)	—	11·1 1 (1)	—	5·0 1 (1)	—	—	—	61·9 12·3 (2–58)	—	13·6 1 (1)	26·1 1·33 (1–3)	—	—
Trematoda																
Diplostomum sp. larv.	lens of eye	90·5 6·42 (1–18)	90·5 15·95 (1–45)	80·9 8·35 (3–38)	38·9 2·43 (1–5)	30·0 2·5 (1–7)	25·0 1·2 (1–2)	—	22·7 2 (1–5)	15·0 2·67 (1–6)	—	21·1 2·75 (1–5)	—	—	—	—
Tylodelphys sp. larv.	Humour of eye	9·5 6 (3–9)	19·1 14·25 (3–24)	—	—	10·0 1·5 (1–2)	—	—	4·5 1 (1)	5·0 6 (6)	—	—	—	—	—	—

Table 2 (*Cont.*)

Parasite species/ Population	Micro-habitat	FIN2	FIN1	PL1	CZ3	CZ4	CZ2	CZ1	I1	F1	BG1	F2	I2	BG2	E1	E2
Apharyngostrigea sp. larv.*	M	—	—	—	—	—	—	—	9·1 12 (1–23)	—	—	—	—	—	—	—
Rhipidocotyle campanula larv. (Dujardin, 1845)	G, F, M	52·4 43·54 (1–190)	9·5 4·5 (2–7)	—	94·4 192·46 2–2000	—	—	—	—	—	100 142·71 (4–495)	—	—	4·3 3 (3)	—	—
Ichthyocotylurus platycephalus larv. (Creplin, 1852)*	M	4·8 2 (2)	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Posthodiplostomum sp. larv.	S, M	—	—	100 20·43 (8–48)	—	—	—	—	—	—	4·8 1 (1)	—	—	—	—	—
Bucephalus polymorphus larv. Baer, 1827	G, ST	—	—	—	94·4 96·23 2–1000	—	—	—	—	—	4·8 1 (1)	—	—	—	—	—
Metagonimus yokogawai larv. Katsurada, 1912*	F	—	—	—	—	—	—	15·9 1·67 (1–3)	—	—	—	—	—	—	—	—
Apharyngostrigea cornu larv. (Zeder, 1800)*	M	—	—	—	—	—	—	5·2 1 (1)	—	—	—	—	—	—	—	—
Metorchis intermedius larv. Heinemann, 1937	M, G	—	—	61·9 3·15 (1–7)	—	—	—	68·4 15·69 (1–71)	—	—	—	—	—	—	—	—
Paracoenogonimus ovatus larv. Katsurada, 1914*	G	—	—	—	—	—	—	—	—	—	9·5 3 (1–5)	—	—	—	—	—
Sphaerostomum bramae (Müller, 1776)	I	9·5 1 (1)	—	—	—	—	—	31·6 8·67 (1–37)	22·7 1·2 (1–2)	—	—	—	—	—	—	—
Asymphylodora markewitschi Kulakovskaya, 1947*	I	—	—	—	11·1 1·5 (1–2)	—	—	—	—	—	—	—	—	—	—	—

Asymphylodora imitans (Mühling, 1898)*	I	—	—	—	—	—	—	—	4·5 1 (1)	—	—	—	—	—	—	—
Allocreadium isoporum (Looss, 1894)	I	71·4 28·27 (4–145)	38·1 14·5 (1–34)	—	—	—	—	—	—	—	—	—	—	30·4 2·14 (1–4)	—	—
Acanthocephala																
Pomphorhynchus laevis (Müller, 1776)	I	—	—	—	22·2 1 (1)	5·0 1 (1)	10·0 1 (1)	57·9 5·18 (1–15)	90·9 9·05 (1–78)	40·0 3 (1–8)	9·5 10 (2–18)	42·1 8·88 (1–34)	36·4 2·75 (1–5)	73·9 4·65 (1–14)	—	—
Acanthocephalus anguillae (Müller, 1780)	I	—	—	—	—	—	—	21·1 1·25 (1–2)	95·5 25·33 (5–109)	—	14·2 1 (1)	—	22·7 12·6 (2–32)	86·9 4·5 (1–16)	—	—
Acanthocephalus lucii (Müller, 1776)*	I	—	9·5 4 (3–5)	—	—	—	—	—	—	—	—	—	—	—	—	—
Nematoda																
Philometra abdominalis Nybelin, 1928	AC, SSb	—	—	—	—	75·0 5·13 (1–28)	20·0 3·5 (1–8)	—	—	—	—	10·5 1 (1)	—	—	—	—
Philometra sp. larv.	AC, SSb	—	4·8 1 (1)	—	16·7 1·33 (1–2)	60·0 3·67 (1–8)	5·0 1 (1)	—	—	—	—	10·5 2·5 (1–4)	4·5 1 (1)	—	—	—
Philometra rischta Skrjabin, 1923*	F, SSb	—	—	—	—	—	—	—	—	—	—	—	4·5 1 (1)	—	—	—
Raphidascaris acus larv. (Bloch, 1779)	I, AC	—	9·5 1 (1)	—	—	20·0 2 (1–5)	—	—	—	15·0 1·67 (1–2)	—	—	22·7 4 (1–9)	—	—	—
Pseudocapillaria tomentosa (Dujardin, 1843)*	I	—	—	—	—	—	—	5·2 1 (1)	—	—	—	—	—	—	—	—
Rhabdochona denudata (Dujardin, 1845)	I	28·6 3 (1–6)	—	—	—	—	—	—	—	—	42·9 14·22 (1–47)	—	27·3 10·17 (1–33)	30·4 2·71 (1–7)	4·3 1 (1)	—
Cucullanus dogieli Krotas, 1959*	I	4·8 1 (1)	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Camallanus lacustris (Zoega, 1776)	I	4·8 1 (1)	4·8 1 (1)	—	—	—	—	—	—	—	—	—	—	—	—	—
Anguillicola crassus larv. Kuwahara, Niimi et Itagaki, 1974*	SSb, AC	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4·7 1 (1)

Table 2 (*Cont.*)

Parasite species/ Population	Micro-habitat	FIN2	FIN1	PL1	CZ3	CZ4	CZ2	CZ1	I1	F1	BG1	F2	I2	BG2	E1	E2
Schulmanela petruschewskii (Schulman, 1948)*	L	—	—	—	—	—	—	—	—	—	—	—	—	—	4·3 2 (2)	—
Nematoda sp.*	AC	—	—	—	—	—	—	—	—	—	—	—	—	—	8·7 1·5 (1–2)	—
Cestoda																
Proteocephalus sp.*	I	—	—	—	—	—	35·0 4·14 (1–12)	—	—	—	—	—	—	—	—	—
Proteocephalus sp. juv.	I	—	—	—	5·8 2 (2)	—	15·0 4·3 (2–7)	—	—	—	—	—	—	—	—	—
Proteocephalus torulosus (Batsch, 1786)	I	—	—	—	22·2 2·25 (1–4)	—	15·0 2·6 (1–6)	5·2 1 (1)	—	—	—	—	—	—	—	—
Caryophyllaeus laticeps (Pallas, 1781)	I	—	—	—	—	—	—	—	—	—	—	26·3 3·6 (2–7)	—	4·3 5 (5)	—	—
Caryophyllaeus brachycollis Janiszewska, 1951	I	—	—	—	—	—	—	5·2 7 (7)	—	—	—	21·1 3·6 (1–9)	—	17·4 1·25 (1–2)	—	—
Caryophyllaeides fennica (Schneider, 1902)	I	—	—	—	—	—	—	21·1 3·25 (1–9)	—	—	—	5·3 1 (1)	—	—	—	—
Caryophyllaeidae sp. larv.	I	—	—	—	—	—	—	—	—	—	4·8 1 (1)	36·8 10·29 (3–33)	—	—	—	—
Caryophyllaeus sp.	I	—	—	23·8 1 (1)	—	—	—	—	—	—	—	21·1 7 (1–14)	—	—	—	—
Cestoda sp.*	I	—	—	—	—	—	—	—	—	—	—	—	13·6 1 (1)	—	—	—

F-fins, M-musculature, G-gills, I-intestine, S-skin, AC-abdominal cavity, SSb-serosa of swimbladder, ST-subcutaneus tissue, BS-body surface, L-liver.

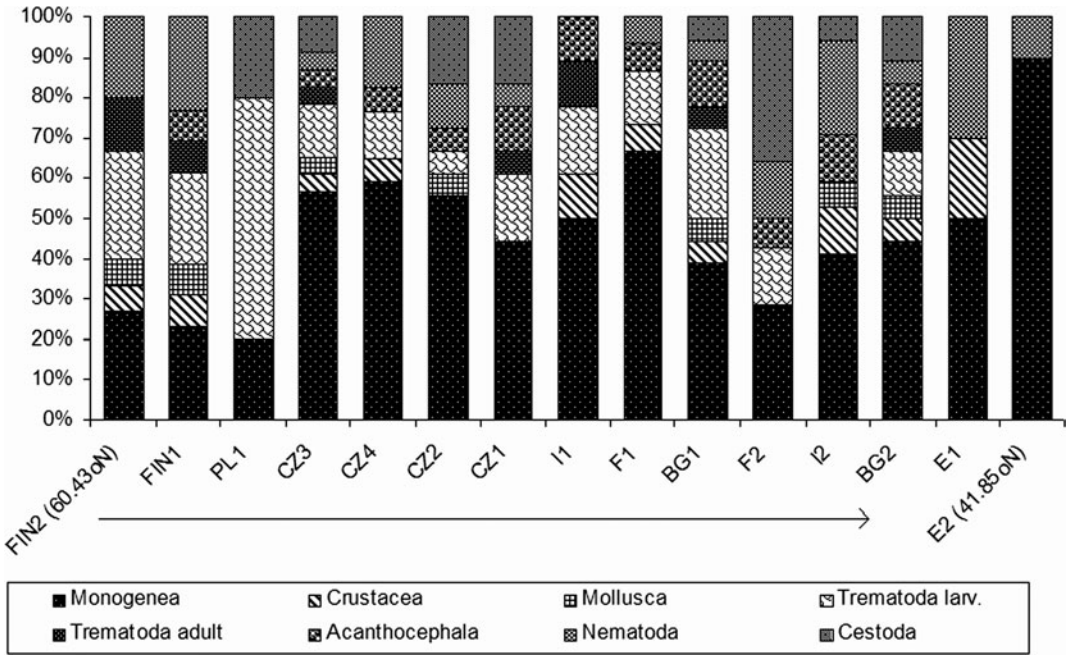

Fig. 2. The proportion of different metazoan groups in the 15 localities studied. Populations are presented according to latitude.

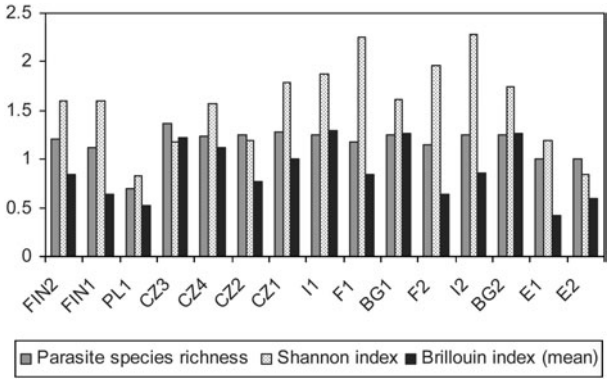

Fig. 3. Comparison of parasite diversity among localities including parasite species richness (log-transformed), Shannon diversity index for each component community and mean parasite infracommunity diversity estimated by Brillouin index. Populations are presented according to latitude.

Infracommunity species diversity varied significantly among localities (KW test, $P<0{\cdot}0001$). Multiple comparisons of mean ranks for all groups revealed statistically significant differences between marginal and central localities ($P<0{\cdot}05$). A general trend of low species diversity in infracommunities was recorded in chub from areas of marginal distribution (localities E1, E2 and PL1) (Fig. 3). Highest parasite species richness and abundance in the component community occurred at locality CZ3 and locality BG1 respectively. The lowest parasite species richness and abundance occurred at locality PL1 and locality E1 respectively. A trend of low species diversity in the component community was also recorded in chub from areas of marginal distribution (Fig. 3).

Relationships of chub populations in Europe

A total of 22 haplotypes were identified. The TrN+G model was selected as the evolutionary model by ModelTest using the following parameters: substitution rate matrix A–C=1·0000, A–G=91·3230, A–T=1·0000, C–G=1·0000, C–T=12·7209, G–T=1·0000 and the rate of heterogeneity approximated by a gamma distribution, $\alpha=0{\cdot}0172$. The phylogenetic tree obtained from all reconstructions showed that all 22 haplotypes of chub found in this study belonged to 4 previously defined phylogenetic lineages (*cf.* Durand *et al.* 1999), i.e., the Aegean, Eastern, Western and Adriatic lineages (Fig. 4), with the following geographical structure: the Adriatic and Eastern lineages had a continuous distribution, Italian populations belonged to the Adriatic lineage, and Polish and Finnish populations belonged to the Eastern lineage. The populations collected in Central Europe (Czech populations), in the south of France and in the Danube River in Bulgaria belonged to the Western lineage. Two haplotypes from the Struma River in Bulgaria and one haplotype from Spain belonged to the Aegean lineage, being a surprising result in the present study.

Diversity and similarity in parasite communities between fish populations: testing the biogeographical hypotheses

Similarity in the composition of parasite communities between pairs of chub populations was computed using the Jaccard and the Morisita indices. Values of both indices ranged from 0 to 1; no similarity was observed between the localities I2 and PL1 (0), the

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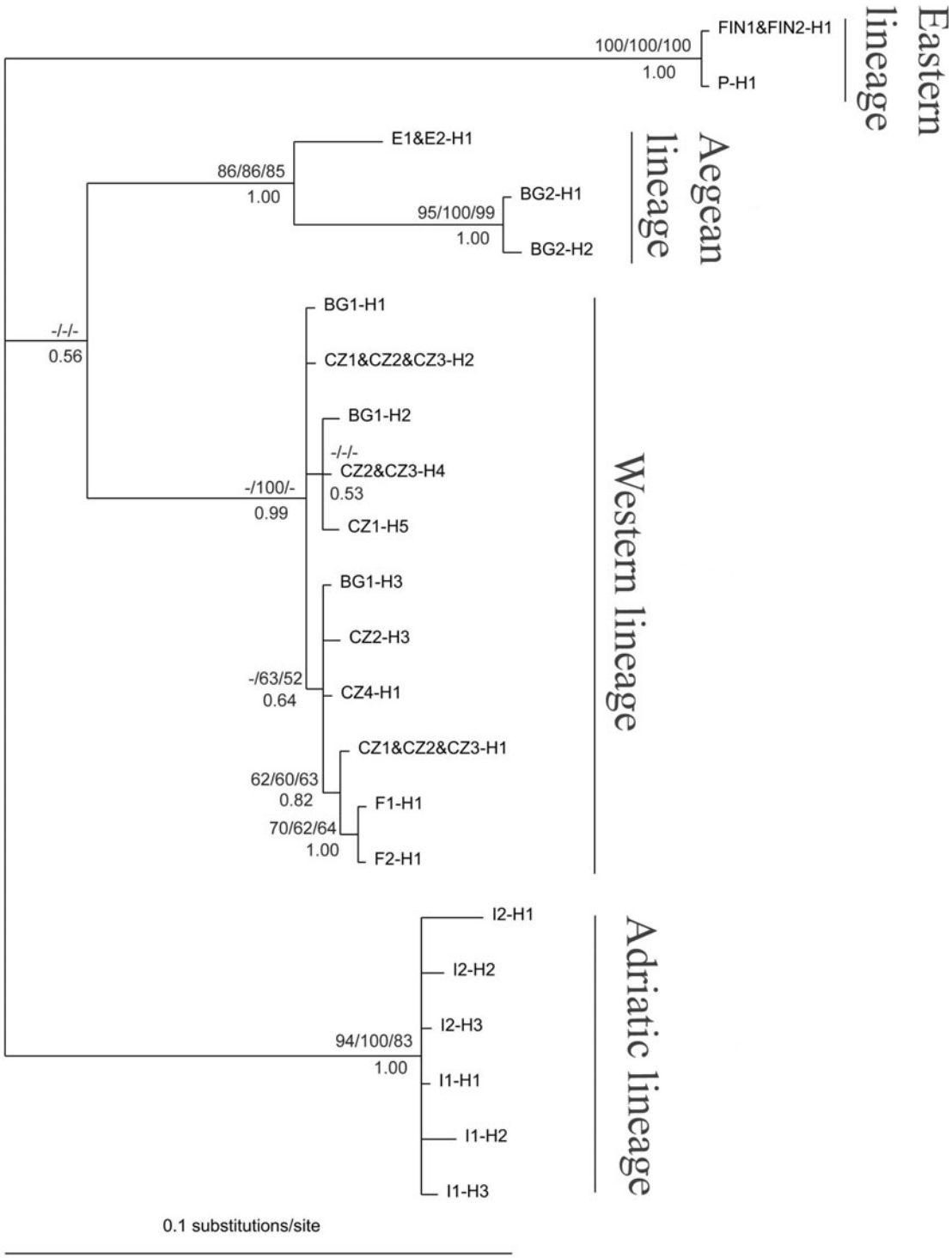

Fig. 4. Consensus Bayesian topology for 22 cytochrome *b* haplotypes. Numbers below branches indicate Bayesian posterior probabilities and numbers above branches indicate bootstrap proportions resulting from the following analyses: ML/MP/NJ.

highest values of the Jaccard index of similarity were obtained between localities belonging to the same phylogenetic lineages: CZ4 and F1 (0·684), CZ3 and CZ2 (0·576), FIN1 and FIN2 (0·526). The highest value of the Morisita index of similarity was between localities CZ3 and BG1 (0·838). There was less similarity in presence/absence data (i.e. Jaccard index) at localities from different latitudes, especially when comparing high and low latitudes, than at localities from the same latitude.

(*a*) *Effects of sample size and host body size on parasite diversity*. No significant correlation between host sample size and parasite species richness or abundance ($P > 0{\cdot}05$) was found among the localities. No correlation between host body size and parasite

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Table 3. The statistics for meta-analyses performed in this study

(The effect size, 95% confidence interval for the point estimate, heterogeneity among species evaluated by Q-statistics and *P*-value are included.)

	The analysed effect	Effect size	95% confidence interval	Q-statistics	*P*-value
Parasite groups	geographical distance × abundance	0·31	0·03, 0·55	3·56	0·61
	phylogenetic distance × abundance	0·39	0·12, 0·61	1·81	0·87
Parasite species	geographical distance × abundance	0·29	0·03, 0·51	5·67	0·84
	geographical distance × prevalence	0·33	0·08, 0·54	7·92	0·64
	phylogenetic distance × abundance	0·44	0·21, 0·63	14·43	0·15
	phylogenetic distance × prevalence	0·38	0·14, 0·58	11·66	0·31
	latitude × abundance	0·21	0·04, 0·37	6·56	0·77
	water temperature × abundance	0·18	0·01, 0·34	1·8	0·99

species richness or abundance ($P>0·05$) was found. Sample size and host body size had no effect on the structure of parasite communities.

(*b*) *Effect of climate and latitudinal gradients on parasite diversity*. All 15 populations were used to examine the potential effects of climate (i.e., effect of water temperature) and latitudinal gradients. A positive correlation was found between latitude and water temperature ($R=0·607$, $P<0·05$). Spearman's rank correlation revealed a significant negative relationship between water temperature and species richness of Monogenea ($R=-0·589$, $P=0·021$) and between water temperature and abundance of the monogenean *D. nanoides* ($R=-0·633$, $P=0·011$). No correlation was found between water temperature and total endoparasite species richness or species richness within each endoparasitic group ($P>0·05$). A positive correlation was found between water temperature and the abundance of the nematode *Rhabdochona denudata* ($R=0·633$, $P=0·010$). No latitudinal gradient was observed for monogenean species richness ($P>0·05$), but there was a negative correlation between latitude and abundance of 1 monogenean species, *D. vistulae* ($R=-0·529$, $P=0·042$). In contrast, a latitudinal gradient was observed for species richness of Trematoda ($R=0·59$, $P=0·020$). When analysing abundance of endoparasite species, a latitudinal gradient was observed for the abundance of metarcercariae of *Diplostomum* sp. ($R=0·839$, $P<0·0001$). No overall significant effects of latitude or water temperature on parasite abundance were found using a meta-analytical approach (Table 3). However, a significant correlation was found between the abundance of metarcercariae of *Diplostomum* sp. and latitude ($P=0·007$) using a meta-analysis, but no parasite abundance was significantly correlated with water temperature ($P>0·05$).

(*c*) '*Favourable centre*' *vs* '*local oasis*' *model*. The first analysis was conducted to test the 'favourable centre' model for each parasite group. A positive correlation between geographical and phylogenetic distances, including all pairs of 15 localities, was found ($R=0·546$, $P<0·0001$). A negative correlation between abundance and both geographical and phylogenetic distances was observed as a general trend for all parasite groups, but a significant correlation was shown only for Monogenea ($P<0·05$). The statistics for meta-analysis is shown in Table 3. No overall significant correlation was found between abundance of parasite groups and geographical distance or phylogenetic distance (Tables 3 and 4). Similarly, no overall correlation was found between abundance or prevalence of parasite species and either geographical distance or phylogenetic distance (Tables 3 and 5). We found only a significant effect of geographical distance on abundance for the case of Monogenea (Table 4). Negative significant correlations were also found between the geographical distance and both abundance and prevalence for the case of *Diplostomum* species using correlation analyses ($P<0·05$) or meta-analysis (Table 5). Both prevalence and abundance of *Diplostomum* sp. were also significantly correlated with phylogenetic distance using correlation analyses ($P<0·05$) or meta-analysis (Table 5). Using a partial correlation coefficient (i.e., both geographical distance and abundance or prevalence were corrected for phylogeny), no significant correlation was found between geographical distance and abundance or prevalence ($P>0·05$). However, a significant negative correlation ($P<0·05$) was observed between phylogenetic distance and both abundance ($R=-0·910$) and prevalence ($R=-0·810$) when correcting for geographical distance.

(*d*) *Factors affecting the similarity of parasite communities*. Analyses were also conducted to evaluate the potential links between latitude, water temperature, geographical distance, host phylogenetic distance and the similarity in parasite communities based on presence/absence (Jaccard index) or abundance data (Morisita index). After transforming matrix data to the vectors for similarity in parasite communities, geographical distance and

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Table 4. The correlation coefficient between abundance and both geographical and phylogenetic distances calculated for each parasite group

(The significant correlations are shown in bold and correspond to the observed correlation coefficient lower than fixed *P*-value from the meta-analysis (Z-value (*P*-value) >2·184 (0·029) for geographical distances, Z-value (*P*-value) >2·774 (0·006) for phylogenetic distances.)

Abundance of parasite group	*n*	Geographical distance (*P*-value)	Phylogenetic distance (*P*-value)
Monogenea	14	**−0·663 (0·008)**	−0·575 (0·030)
Crustacea	9	−0·311 (0·431)	−0·577 (0·107)
Acanthocephala	10	−0·120 (0·750)	−0·342 (0·346)
Trematoda	11	0·246 (0·478)	0·261 (0·450)
Nematoda	12	−0·070 (0·833)	−0·152 (0·646)
Cestoda	7	−0·113 (0·820)	−0·346 (0·470)

Table 5. The correlation coefficient between abundance (Ab) or prevalence (Pr) and both geographical and phylogenetic distances calculated for selected parasite species

(The significant correlations are shown in bold and correspond to the observed correlation coefficient lower than fixed *P*-value from the meta-analysis. Abundance: Z-value (*P*-value) >2·205 (0·027) for geographical distances, Z-value (*P*-value) >3·540 (0·001) for phylogenetic distances. Prevalence: Z-value (*P*-value)>2·590 (0·010) for geographical distances, Z-value (*P*-value) >2·983 (0·003) for phylogenetic distances.)

Parasite species		*n*	Geographical distance (*P*-value)	Phylogenetic distance (*P*-value)
Gyrodactylus vimbi	Ab	13	−0·167 (0·594)	−0·256 (0·408)
	Pr		−0·167 (0·594)	−0·315 (0·302)
Dactylogyrus vistulae	Ab	12	−0·112 (0·736)	−0·152 (0·646)
	Pr		0·089 (0·789)	−0·233 (0·476)
Dactylogyrus folkmanovae	Ab	12	−0·600 (0·038)	−0·296 (0·360)
	Pr		−0·447 (0·149)	−0·157 (0·635)
Dactylogyrus prostae	Ab	9	−0·108 (0·791)	−0·362 (0·363)
	Pr		−0·279 (0·483)	−0·438 (0·250)
Dactylogyrus nanoides	Ab	5	−0·285 (0·678)	−0·654 (0·269)
	Pr		−0·139 (0·843)	0·131 (0·852)
Dactylogyrus vranoviensis	Ab	6	−0·001 (0·999)	−0·549 (0·285)
	Pr		0·124 (0·829)	−0·432 (0·423)
Paradiplozoon megan	Ab	7	0·095 (0·849)	−0·558 (0·208)
	Pr		−0·236 (0·630)	−0·102 (0·838)
Diplostomum sp.	Ab	8	**−0·771 (0·022)**	**−0·962 (0·0001)**
	Pr		**−0·900 (0·0001)**	**−0·948 (0·0001)**
Pomphorhynchus laevis	Ab	9	−0·013 (0·975)	−0·122 (0·764)
	Pr		−0·168 (0·678)	−0·206 (0·609)
Acanthocephalus anguillae	Ab	4	−0·275 (0·778)	−0·732 (0·351)
	Pr		0·346 (0·718)	−0·121 (0·903
Rhabdochona denudata	Ab	4	−0·438 (0·639)	−0·005 (0·991)
	Pr		−0·455 (0·623)	0·184 (0·852)

phylogenetic distance, Spearman's rank correlations were computed between similarity in parasite communities and each variable studied. Significant negative correlations were found between the similarity based on presence/absence data (Jaccard index) and geographical distance (R = −0·292, *P* = 0·0025, Fig. 5A), phylogenetic distance (R = −0·543, *P* < 0·0001, Fig. 6A), latitude (R = −0·321, *P* = 0·0008) and water temperature (R = 0·247, *P* = 0·011). Similarly, negative correlations were found between similarity based on abundance data (Morisita index) and geographical distance (R = −0·300, *P* = 0·0019), (Fig. 5B), phylogenetic distance (R = −0·431, *P* < 0·0001), (Fig. 6B)

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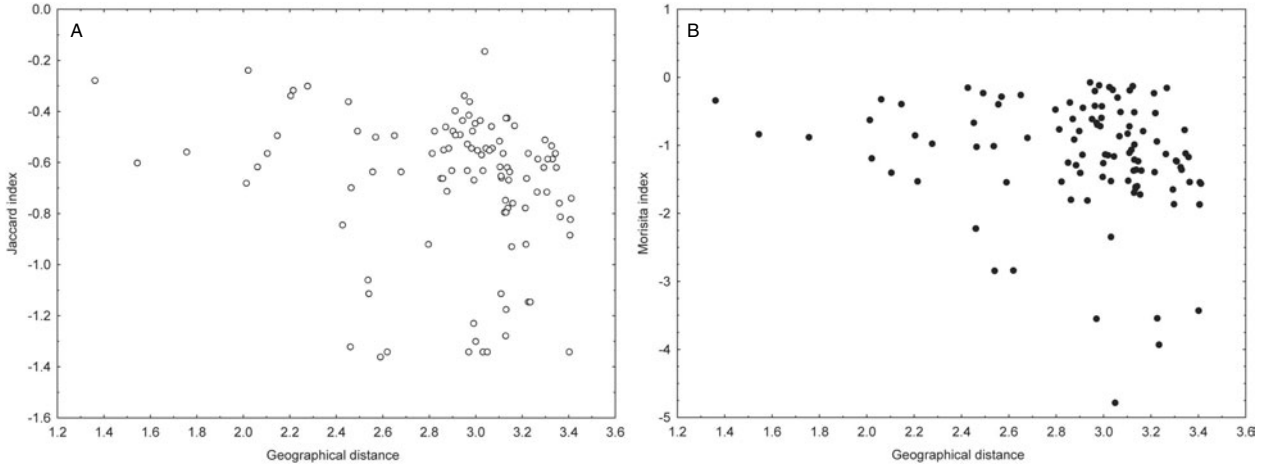

Fig. 5. Negative correlations between geographical distance and similarity in parasite communities calculated using Jaccard index (A) or Morisita index (B) between pairs of localities. Data are log-transformed.

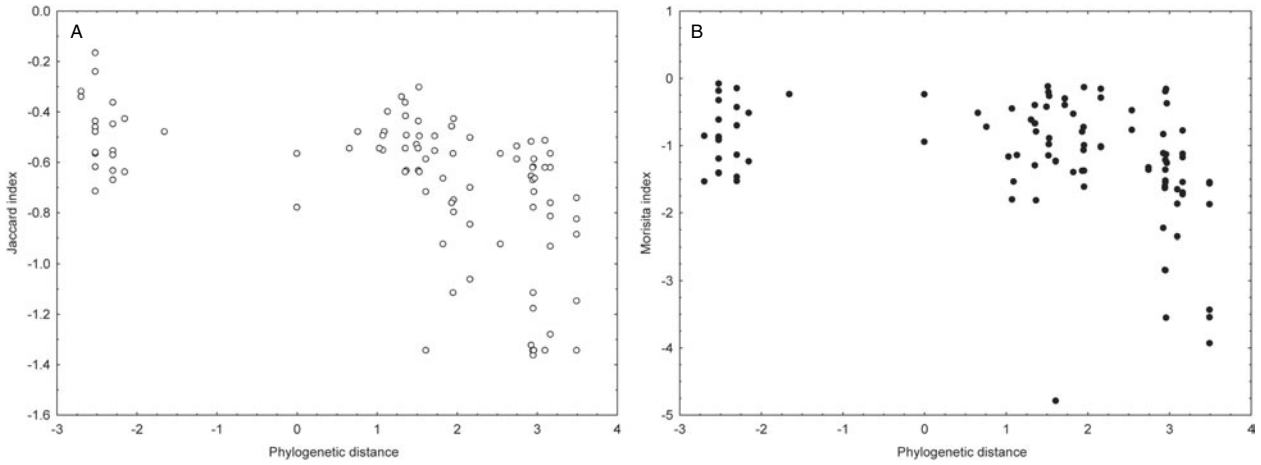

Fig. 6. Negative correlations between phylogenetic distance and similarity in parasite communities calculated using Jaccard index (A) or Morisita index (B) between pairs of localities. Data are log-transformed.

and latitude ($R = -0{\cdot}392$, $P < 0{\cdot}0001$). Multiple regression analysis with 'backward elimination', calculated using permutation methods, showed that only host phylogeny made a statistically significant contribution to the qualitative and quantitative similarities of parasite communities (for Jaccard index: $N = 105$, $b = -0{\cdot}448$, $R^2 = 0{\cdot}193$, $P = 0{\cdot}002$; for Morisita index: $N = 105$, $b = -0{\cdot}323$, $R^2 = 0{\cdot}105$, $P = 0{\cdot}001$). Similarity in parasite communities decreased significantly with increasing phylogenetic distance between host populations, whereas water temperature, latitude and geographical distance had no significant effect on the similarity of parasite communities.

DISCUSSION

We examined the composition of metazoan parasite communities of chub (*Leuciscus cephalus*) from European rivers at 15 localities, covering a wide geographical range of chub distribution to elucidate the potential roles of temperature, latitude, geographical and phylogenetic distances in determining ecto- and endo-parasite diversity and community composition.

The metazoan parasite fauna consisted of 8 groups, with monogeneans and trematodes representing the most numerous taxa. No parasite species occurred at all localities, and 22 species (comprising 35·5% of total parasite species) were characteristic for only a single locality. Fish in localities in marginal zones of chub distribution had lower species diversity in both infracommunities and component communities. Highest species richness occurred in a Czech locality, Labe, where 13 species of monogeneans were recorded. The lowest species richness was found in a Polish locality, Mroga, where only 1 monogenean species occurred.

We compared the potential effect of latitude on species richness of ecto- *versus* endo-parasitic groups and the abundance of selected parasite species. There was a significant correlation between latitude and

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species richness of trematodes. Moreover, we recorded the correlation between latitude and abundance of *Diplostomum* sp. (the most prevalent endoparasite species). Abundance of this species increased northward, whereas only the abundance of the monogenean *D. vistulae* increased significantly southwards. Latitudinal gradients in species richness are well documented for parasites (Rohde and Heap, 1998; Poulin and Dick, 2007; Krasnov *et al.* 2008). An increase in species diversity towards tropical areas is the result of higher rates of evolution or effective evolutionary time, which are correlated with temperature (Rohde, 1992). Large areas with a tropical climate represent regions with a lower risk of extinction and higher speciation rates (Rosenzweig and Sandlin, 1997). Rohde *et al.* (1995) observed that marine fish in tropical seas typically harbour richer ectoparasite communities than fish from higher latitudes. Latitudinal gradients have been observed for genera of Monogenea and Trematoda and for species within the Monogenea (Rohde, 1984). In several studies, there was no support for the latitudinal gradient (or temperature gradient) hypothesis for ectoparasite species richness in marine fishes (e.g., González and Moreno, 2005). Among freshwater fish species, greater helminth species richness has been reported at temperate latitudes than in the tropics (Choudhury and Dick, 2000; Poulin, 2001), possibly because fish in temperate waters tend to be larger in size than those in tropical waters (Poulin, 2001). We observed latitudinal gradients in highly prevalent species, such as Monogenea and Trematoda. However, while the monogenean *D. vistulae* decreased in abundance towards higher latitudes, the opposite was shown for metacercariae of *Diplostomum* sp. as well as total parasite species richness of Trematoda. An indirect latitudinal effect (i.e., for water temperature) was found for the nematode *R. denudata*. However, it is emphasized that, after performing a meta-analysis, the latitudinal gradient was found only for *Diplostomum* species and no temperature effect was found for abundance of any parasite species. A positive relationship between latitude and prevalence of endoparasite species (i.e. *Proteocephalus pearsei* in *Perca flavescens*) was documented by Poulin and Dick (2007), suggesting that this gradient could generate a geographical distribution with a centre of high prevalence but may also reflect the distribution of intermediate hosts for those endoparasites with indirect life cycles. The effect of the presence of intermediate hosts on endoparasite diversity has been documented in several studies (Poulin, 1995; Lile, 1998). In the present study, the finding of a positive correlation between latitude and water temperature as well as a negative correlation between species richness in Monogenea and water temperature should be interpreted with some caution. Although the sampling herein aimed to collect fish and parasite samples at approximately the same time of year, to exclude potential seasonal variability, we could not exclude temporal variability in water temperature associated with specific characteristics of a given year or locality, particularly in relation to recent climatic changes, such as the beginning and end of spring and summer or temperature fluctuations associated with anthropogenic activities. Mean annual temperature (not available for the present study) may represent a better estimator for testing temperature effect in subsequent studies. The absence of a latitudinal gradient in relation to Monogenea may be explained by a higher monogenean species diversity and abundance in the central regions than in the marginal zones of chub distribution documented in this study.

The 'favourable centre' model hypothesis assumes that species abundance decreases from the centre of the species range towards its margins (Sagarin and Gaines, 2002). González and Moreno (2005) observed lower ectoparasite species richness in marginal areas of distribution of the marine fish *Sebastes capensis*. These authors also suggested that the low density of host fish and/or their limited movements in marginal zones would result in limited contact between infected and uninfected fish, while fish populations in central zones would be much more interactive, ensuring higher infestation rates. The 'favourable centre' hypothesis is supported by the phylogenetic relationships found in mitochondrial haplotypes among Czech and French populations of chub and one Bulgarian population, areas considered to be at the geographical centre of chub distribution, suggesting possible historical contacts.

Poulin and Dick (2007) tested the prediction of the 'favourable centre' model using 8 helminth species of freshwater fish, *Perca flavescens*, including species of endoparasitic Trematoda, Cestoda, Nematoda and Acanthocephala. Only the prevalence of the cestode *Proteocephalus pearsei* was correlated negatively with the distance from the locality with maximum prevalence. Both abundance and prevalence of this cestode species increased with latitude. Overall parasite abundance in perch did not support the prediction of the 'favourable centre' model. Consequently, a decrease in prevalence of *P. pearsei* with the decreasing distance from the locality with maximum prevalence was considered a possible result of a latitudinal gradient effect in prevalence, because this parasite species exhibited highest prevalence in the northernmost localities (Poulin and Dick, 2007). In contrast, Sagarin and Gaines (2002) found that 56 studies from 145 separate tests supported the 'abundant centre' hypothesis, although only two studies analysed data collected throughout the geographical range of the host species. Like Poulin and Dick (2007), we found no general support for the 'favourable centre' model, because the majority of correlations were not significant. However,

for all parasite groups or species, there was a trend for a negative correlation between prevalence and/or abundance and geographical distance from the locality with maximum prevalence or abundance. This trend was identical for 7 of 11 species analysed using abundance or prevalence data. Significant correlations were calculated only for total abundance of Monogenea, abundance of the monogenean *D. folkmanovae* and both abundance and prevalence of the trematode *Diplostomum* species. The highest prevalence of *Diplostomum* sp. occurred in northernmost localities, and its abundance correlated positively with latitude, as reported for the cestode *P. pearsei* (see Poulin and Dick, 2007). Parasite abundance was higher in localities closest to the centre of maximum abundance for total Monogenea as well as for *D. folkmanovae*. However, after performing a meta-analysis, the correlation was not significant for *D. folkmanovae*. The 'favourable centre' hypothesis may be applicable particularly to highly specific monogenean parasites, because abundance values are not affected by potential distribution of intermediate hosts or other potential definitive hosts, as in the case of many generalist endoparasite species. This proposal should be further explored in future using different monogenean models, together with an assessment of phylogenetic affinities of the host populations investigated. In the present study, we showed a significant positive correlation between geographical and phylogenetic distances and a negative but non-significant correlation between abundance or prevalence and phylogenetic distance from the locality with maximum abundance or prevalence of parasite species. Thus, the 'favourable centre' hypothesis seems to be driven more by phylogenetic distance between host populations than geographical distance; alternatively, the 'favourable centre' hypothesis was supported only indirectly due to the positive correlation between geographical and phylogenetic distances. When both abundance and geographical distance were corrected for phylogenetic distance using partial correlation, no significant relationship was observed.

The final hypothesis tested was that of decay of similarity with distance in parasite communities. The decreasing proportion of species shared by two communities with increasing distance is not only obvious in free-living organisms (Nekola and White, 1999), but 'distance decay' in species composition is also common in parasites. This pattern has been documented in parasite communities of freshwater and marine fishes (Poulin, 2003; Fellis and Esch, 2005; Oliva and González, 2005). In the present study, the similarity in parasite communities was studied using the Jaccard qualitative index and the Morisita quantitative index. A low similarity was found between northern and southern localities, but the highest values of similarity using both indices were recorded primarily between localities belonging to the Western lineages. According to Faith *et al.* (1987), the Jaccard index is more effective in detecting underlying ecological gradients. On the other hand, abundance data may be affected more by local conditions than by geographical distance (Fellis and Esch, 2005). Therefore, presence/absence data are more appropriate to elucidate the influence of geographical distance on parasite similarity. The decay of similarity with distance has several potential explanations, such as a decrease in environmental similarity with distance, spatial configuration, limited dispersion, niche width differences among taxa, or, it may be consistent with Hubbell's (2001) neutral theory.

Because the evolutionary history of the host species may affect the composition of the parasite community, phylogenetic distance between fish populations were included in the analysis of decay of similarity with distance in parasite communities. Using phylogenetic reconstruction, based on cytochrome *b* sequence datasets for 4 different evolutionary lineages (Western, Adriatic, Aegean and Eastern lineages; Durand *et al.* 2000), the existence of 4 contemporary lineages was reported and attributed to rapid radiation (dispersion and fragmentation events) by Durand *et al.* (2000), the current geographical distribution of chub is a result of the post-glacial dispersion of the species in Europe; thus, the phylogeography of chub may be related to parasite diversity, particularly for highly specific parasites. Generally, populations belonging to the Western, Adriatic and Aegean lineages exhibited higher species diversity. Populations of the Eastern lineage exhibited low parasite species diversity. However, other factors, such as water temperature and latitude or local ecological factors (not investigated in this study), could be considered as important determinants affecting the similarity in parasite communities. Using a simple correlation, we showed that all 4 variables were significantly related to similarity in parasite communities. Such a correlation between geographical distance and similarity in parasite communities may indicate support for the 'distance decay' hypothesis. However, permutation of distance data indicated that only host phylogeny contributed significantly to similarity between parasite communities. This finding emphasizes the importance of phylogenetic information on host populations when testing for decay of similarity with distance in parasite communities. To date, no study investigating the 'distance decay' hypothesis in parasites has examined the potential effect of relatedness in host populations, despite many such studies being performed on hosts with a broad geographical distribution. Similarly, in free-living organisms, the history of the geographical areas investigated should represent an important factor affecting the decay of species similarity with geographical distance.

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