Comparative phylogeography of two monogenean species (Mazocraeidae) on the host of chub mackerel, *Scomber japonicus*, along the coast of China

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SUMMARY

In the present paper, the phylogeographies of two monogenean species, *Pseudokuhnia minor* and *Kuhnia scombri*, on the same species of host, *Scomber japonicus*, were studied. Fragments of the mitochondrial cytochrome c oxidase subunit 1 gene were sequenced for 264 individuals of *P. minor* and 224 individuals of *K. scombri* collected from 10 localities along the coast of China. Genetic diversity of *K. scombri* was higher than that of *P. minor*, which may imply that *P. minor* has a lower evolution rate and/or is a younger species. The neighbour-joining (NJ) trees of both parasites were comprised of two clades without association to sample sites, which is the signature of remixing populations following past division. Analyses of molecular variance and pairwise fixation index revealed different genetic structures for the populations of these two closely related species along the coast of China: *P. minor* without significant genetic structure, while *K. scombri* has some genetic differentiation. Both neutrality tests and mismatch distribution suggested that the populations of these two species of parasites experienced population expansion in the late Pleistocene era due to the glacial–interglacial cycles induced by climatic oscillations.

Key words: Monogenea, chub mackerel, mitochondrial DNA, COI, phylogeography, coast of China.

INTRODUCTION

Phylogeography targets the principles and processes determining the geographical distribution of genetic lineages at the intraspecific or congeneric levels, which is useful for understanding processes such as population subdivision, speciation and ecological adaptation to past climatic changes (Avise, 2000). Historical process associated with climatic oscillations in the Pleistocene ice ages is one of the most important factors in determining the current distribution of marine organisms (Avise, 2000). The Western Pacific continental margin accounts for more than 75% of the marginal basins in the modern global sea (Tamaki and Honza, 1991). During late Pleistocene glacial cycles, the sea-level-induced environmental alterations were amplified in the marginal seas of Western Pacific, leading to drastic changes in areas and configurations of these seas (Wang, 1999), which renders the Northwestern Pacific Ocean an ideal system for understanding the role of sea-level fluctuations during the Pleistocene era in shaping phylogeographic patterns of marine organisms. Numerous studies conducted in this area have concentrated on widely distributed free-living organisms such as fishes (Han *et al.* 2012), mollusks (Niu *et al.* 2010), crustaceans (Wang *et al.* 2008) and mammals (Chen *et al.* 2010). Parasites, as a result of their peculiar life cycle and their close physical associations with their hosts, were often considered to have higher evolutionary potential than that of free-living organisms (Huyse *et al.* 2005), and are expected to have unique phylogeographical patterns (Plaisance *et al.* 2007). Monogeneans, which mainly parasitize fishes (Zhang *et al.* 2001), were considered the ideal subjects for investigation of the dynamics and mechanism of co-evolution between parasites and their hosts due to their direct life cycle (no need of intermediate hosts) and high degree of host specificity (Pariselle *et al.* 2011). However, reports of studies on the phylogeography of monogeneans are still very limited (Li *et al.* 2011; Shi *et al.* 2014).

There have been comparative studies on phylogeographies of monogeneans, either different species of monogeneans on different species of hosts (Plaisance *et al.* 2007) or the same species of monogeneans on different species of hosts

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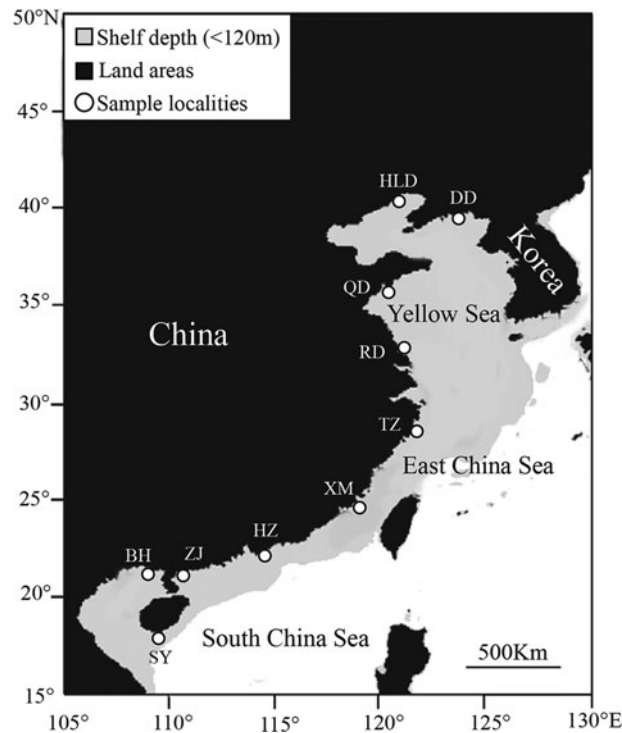
Fig. 1. Map showing sample localities for *Pseudokuhnia minor* and *Kuhnia scombri*. Shaded areas indicate continental shelves that would have been exposed during periods of low sea level. HLD, Huludao; DD, Dandong; QD, Qingdao; RD, Rudong; TZ, Taizhou; XM, Xiamen; HZ, Huizhou; ZJ, Zhanjiang; BH, Beihai; SY, Sanya. This figure was modified after Liu *et al*. (2006).

(Glennon *et al*. 2008). To date, no study on phylogeographies of different species of monogeneans on the same species of hosts has been reported.

During our investigation of fish monogeneans along the coast of China, we found that two species of monogeneans, *Pseudokuhnia minor* and *Kuhnia scombri*, on the host, *Scomber japonicus*, were widely distributed. We therefore carried out research to compare the phylogeographies of these two species of monogeneans on the same species of host along the coast of China. Based on the sequences of mitochondrial cytochrome c oxidase subunit 1 (COI) gene of both parasites from ten localities along the coast of China, we aimed to: (1) test the genetic diversity and demographic history of these two species of monogeneans; (2) compare the phylogeographic patterns and genetic structure of these two sympatric monogeneans on the same host species; and (3) propose scenarios of population expansion and recolonization of these two parasites.

MATERIALS AND METHODS

Sample collection

A total of 264 specimens of *P. minor* and 224 specimens of *K. scombri* on the host of *S. japonicus* were collected from 10 localities along the coast of China from August 2011 to November 2013. Sample locations are as follows: Huludao (HLD, 40°50′N, 121°05′E), Dandong (DD, 39°45′N, 124°08′E), Qingdao (QD, 36°04′N, 120°20′E) and Rudong (RD, 32°20′N, 121°11′E) in the Yellow Sea (YS); Taizhou (TZ, 28°68′N, 121°43′E) and Xiamen (XM, 24°28′N, 118°06′E) in the East China Sea (ECS); Huizhou (HZ, 23°05′N, 114°24′E), Zhanjiang (ZJ, 21°27′N, 110°35′E), Beihai (BH, 21°20′N, 108°47′E) and Sanya (SY, 18°25′N, 109°50′E; Hainan Island) in the South China Sea (SCS). Detailed geographic locations and sample size are given in Fig. 1 and Table 1. Fish samples were purchased from fishermen and morphologically identified to the species in the temporary field laboratory. Parasites were isolated from the gills and identified at the species level by microscopy based on morphological characteristics (Sproston, 1945; Rohde and Watson, 1985). The identified specimens of parasites, *P. minor* and *K. scombri*, were preserved in 95% ethanol for subsequent DNA extraction and analysis.

DNA extraction, PCR amplification and sequencing

Specimens of each parasite were individually removed from ethanol and placed in Tris EDTA (TE) buffer (100 mM Tris–HCl, 10 mM EDTA) for 30 min, then transferred into deionized water for 10 min to wash away excessive TE buffer. Finally, each individual was put into a 0·2 mL tube containing 19 µL of lysis buffer (Tiangen Biotech, China)

Table 1. *Sampling localities, grouped regions and descriptive statistics of genetic diversity of* Pseudokuhnia minor

Region and site	Abbreviation	N	H	S	h	π	k
Yellow sea	YS	103	99	137	0·9992 ± 0·0015	0·015352 ± 0·007763	11·698323 ± 5·341046
Huludao	HLD	26	26	76	1·0000 ± 0·0107	0·013146 ± 0·006918	10·017158 ± 4·732229
Dandong	DD	25	24	73	0·9967 ± 0·0125	0·016954 ± 0·008807	12·919179 ± 6·021764
Qingdao	QD	26	26	78	1·0000 ± 0·0107	0·014795 ± 0·007730	11·273766 ± 5·287212
Rudong	RD	26	26	67	1·0000 ± 0·0107	0·016859 ± 0·008745	12·846622 ± 5·981552
East China sea	ECS	52	52	105	1·0000 ± 0·0038	0·015728 ± 0·008021	11·984887 ± 5·508521
Taizhou	TZ	26	26	78	1·0000 ± 0·0107	0·014850 ± 0·007757	11·315632 ± 5·305698
Xiamen	XM	26	26	71	1·0000 ± 0·0107	0·016562 ± 0·008599	12·620171 ± 5·881602
South China sea	SCS	109	105	156	0·9992 ± 0·0015	0·016966 ± 0·008528	12·927745 ± 5·867762
Huizhou	HZ	29	29	95	1·0000 ± 0·0091	0·018093 ± 0·009311	13·786490 ± 6·373961
Zhanjiang	ZJ	27	27	75	1·0000 ± 0·0101	0·017829 ± 0·009207	13·585401 ± 6·299673
Beihai	BH	28	28	80	1·0000 ± 0·0095	0·015322 ± 0·007965	11·675446 ± 5·451367
Sanya	SY	25	24	76	0·9967 ± 0·0125	0·016801 ± 0·008731	12·802527 ± 5·970207
Total samples	Total	264	250	202	0·9995 ± 0·0004	0·016061 ± 0·008053	12·238673 ± 5·546383

N, sample size; H, number of haplotypes; S, number of segregating sites; h, haplotype diversity (±S.D.); π, nucleotide diversity (±S.D.); k, mean pairwise difference (±S.D.).

with $1\,\mu g\,\mu L^{-1}$ proteinase K (Tiangen Biotech, China) and incubated at 56 °C for 40 min with shaking, then 94 °C for 10 min to inactivate proteinase K. With the lysate as template, the partial mitochondrial COI was amplified using nested PCR reaction due to the low content of genomic DNA extracted from such a small individual worm. Primers used were as follows: COI_Mono_5: 5′-TAATWGGTGGKTTTGGTAA-3′ (forward) and COI_Mono_3: 5′-TAATGCATMGGAAAAAAA CA-3′ (reverse) for the primary PCR amplifications; COI_ Mono_5 (forward) and COI_Mono_int3: 5′-A CATAATGAAARTGAGC-3′ (reverse) for the nested PCR amplifications (W: A/T; K: G/T; M: A/C; R: A/G), just the same as those used by Plaisance *et al*. (2007). Each PCR reaction was performed in a volume of $50\,\mu L$ containing $5\,\mu L$ of 10 × reaction buffer, $5\,\mu L$ of $MgCl_2$ (25 mM), $1\,\mu L$ of dNTPs (10 mM), 10 pM of each primer, 2·5 units of Ex Taq DNA polymerase (TaKaRa, Dalian, China), $0·25\,\mu L$ of lysate for the primary amplifications, and $0·25\,\mu L$ of original product for the nested amplifications. Both the primary and nested PCR amplification were carried out in a Biometra thermal cycler under the following conditions: 5 min initial denaturation at 94 °C, and 35 cycles of 30 s at 94 °C for denaturation, 30 s at 44 °C for annealing, 80 s at 72 °C for extension, and a final extension at 72 °C for 8 min. The final PCR products were purified using the UNIQ-10 Spi Column PCR Product Purification Kit (Sangon, China) and subjected to automated DNA sequencing (BGI, China) with the same primers used for nested amplification.

Data analyses

All analyses were carried out separately for *P. minor* and *K. scombri*. Each DNA sequence was edited and aligned by MEGA 6.0 (Tamura *et al*. 2013) and further verified by naked eyes, then translated into amino acid sequences using a flatworm mitochondrial code to insure that no nuclear pseudogenes were amplified.

Molecular diversity indices such as number of haplotypes (H), polymorphic sites (S), transitions, transversions and indels were calculated using Arlequin 3.5 (Excoffier and Lishcer, 2010). The same software was used to evaluate parameters of haplotype diversity (h; Nei, 1987), nucleotide diversity (π; Nei, 1987) and the average number of pairwise nucleotide differences (k; Tajima, 1983). Pairwise and overall distances among haplotype sequences were calculated in MEGA 6.0 (Tamura *et al*. 2013).

Modeltest 3.7 (Posada and Crandall, 1998) was used to determine the best substitution model for both parasite species based on the Hierarchical Likelihood Ratio Tests. Consequently, the Tamura and Nei evolutionary model (Tamura and Nei, 1993) with the gamma shape parameter (G = 0·6798 for *P. minor* and G = 1·1714 for *K. scombri*) was selected for both species and applied to the subsequent analyses of phylogenetic relationship and molecular variances [analysis of molecular variances (AMOVA)].

Both neighbour-joining (NJ) (Saitou and Nei, 1987) and Bayesian inference (BI) methods were adopted to reconstruct the phylogenetic tree, by MEGA 6.0 with 1000 bootstrap replicates and MrBayes version 3.2.1 (Ronquist *et al*. 2012) respectively, with *Mazocraeoides gonialosae* (Monogenea, Mazocraeidae) as an outgroup. For well supported clades, the divergence time was estimated from net divergence by the formula $t = d_A/2\mu$ (Nei and Li, 1979), in which μ is the mutation rate of the specific gene, and d_A was obtained by MEGA

using Tamura and Nei model with gamma shape parameter given by Modeltest. In addition, genealogical relationships were examined by constructing haplotype networks in Network 4.6.1.1 (http://www.fluxus-engineering.com) using the median-joining network (MJN) approach (Bandelt *et al.* 1999) with Maximum Parsimony (MP) Calculation (Polzin and Daneshmand, 2003).

Using Arlequin 3.5 (Excoffier and Lishcer, 2010), the AMOVA was performed to characterize population structure by *F*-statistics at three subdivided geographical hierarchical levels: the proportions of variations among regions ($F_{CT'}$), among populations within region ($F_{SC'}$) and within populations ($F_{ST'}$). The three regions as divided represent three marginal seas of China, i.e. the Yellow Sea, the ECS and the SCS. The significance of the covariance components associated with the different levels of genetic structure was determined by a permutation test of 5000 times. Genetic differentiation of paired local populations was evaluated by pairwise fixation index (F_{ST}), which includes information of both mitochondrial haplotype frequency and genetic distances. For each pairwise comparison, the significance of the F_{ST} was tested by 10 000 permutations. To test whether the observed distribution of frequencies coincides with the expectation under panmixia (Raymond and Rousset, 1995), the exact test was performed for the differentiation of haplotypes among different local populations to test the null hypothesis of population panmixia. Probabilities were estimated by permutation analyses using 10 000 randomly permuted *r* (populations) × *k* (different haplotypes) contingency tables of haplotype frequencies.

The historical demographic patterns of *P. minor* and *K. scombri* populations were examined by two different approaches. First, the frequency distribution of pairwise differences among all haplotypes (mismatch distribution) was tested under the sudden expansion model of Rogers (Rogers and Harpending, 1992; Rogers, 1995). The mismatch distribution is usually multimodal in samples drawn from populations at demographic equilibrium, but it is usually unimodal in populations which have undergone recent population expansions in demography and range (Rogers and Harpending, 1992). Past demographic parameter *τ* (time since expansion expressed in units of mutational time) was estimated (Rogers and Harpending, 1992). Deviations from the estimated demographic model were evaluated by the tests of Harpending's raggedness index (Harpending, 1994) and the sum of squared differences (SSD) with a parametric bootstrapping approach using 10 000 replicates. Given that mismatch distributions were generally very conservative (Ramos-Onsins and Rozas, 2002), both Tajima's *D* (1989) and Fu's F_S (1997) tests were adopted to test the neutrality. Tajima's *D* test compares two estimators of the mutation parameter *θ*,

Watterson's estimator *θ*s and Tajima's estimator *θπ*; significance of the *D* value is associated with population expansion, bottlenecks and selection (Tajima, 1989). In Fu's F_S test, the number of haplotypes observed is contrasted with that expected in a random sample under the assumption of an infinite-sites model without recombination (Fu, 1997). F_S is sensitive to demographic expansion, which generally leads to a significant negative value of F_S. Both mismatch analysis and neutrality tests were performed in Arlequin 3.5.

The values of *τ* generated by mismatch distribution analysis were utilized to estimate the time back to the expansion by the equation $τ = 2ut$ (Rogers and Harpending, 1992), in which *u* is the mutation rate of sequence in one generation and calculated by the equation $u = 2 \mu k$, where *μ* is the mutation rate per nucleotide and *k* is the number of nucleotides included in analyses. Finally, the approximate time of expansion in years was obtained by multiplication of *t* and the generation times of *P. minor* and *K. Scombri*, respectively. Neither the generation times nor the mutation rate of the COI gene of these two parasites have been determined; so a generation time of 1 year and the mutation rate of 10% per million years used for *M. gonialosae*, a mazocraeid (Li *et al.* 2011), were used as reference values for the two mazocraeids examined in the present study.

RESULTS

Genetic diversity

Partial sequences of COI gene were obtained from 264 individuals of *P. minor* and 224 individuals of *K. scombri* on the host, *S. japonicus*, sampled in 10 localities along the coast of China (Table 1). All haplotype sequences were deposited in GenBank under accession number KU379830 – KU380079 (*P. minor*) and KU380080 – KU380243 (*K. scombri*).

For the 264 partial sequences of 762 bp of *P. minor*, a total of 202 polymorphic sites including 138 parsimony informative ones were detected, including 191 transitions, 45 transversions and no indels. Sequence divergence (Tamura and Nei distance) among haplotypes ranged from 0·1 to 4·8%, with an average of 1·6%. The average base composition was as follows: *A* = 26·16%, *T* = 38·17%, *C* = 15·83%, *G* = 19·84%, characterized by A+T rich. Among 250 haplotypes defined, with the exception of 10 which are shared by two to four individuals, all others are unique. The haplotype diversities (*h*) were high for all local populations, with the value of 1·000 for 8 of 10 localities and an overall value of 0·9995. However, the nucleotide diversities (*π*) were generally low, ranging from 0·013146 to 0·018093 and an overall value of 0·016061 (Table 1).

The fragment length of COI gene obtained from *K. scombri* was 765 nucleotides, including 134

Table 2. *Sampling localities, grouped regions and descriptive statistics of genetic diversity of* Kuhnia scombri

Region and site	Abbreviation	N	H	S	H	π	k
Yellow sea	YS	93	72	96	0·9857 ± 0·0055	0·023193 ± 0·011511	17·742711 ± 7·949322
Huludao	HLD	27	23	57	0·9858 ± 0·0150	0·020785 ± 0·010656	15·900533 ± 7·319843
Dandong	DD	21	20	57	0·9952 ± 0·0165	0·023901 ± 0·012329	18·284488 ± 8·449453
Qingdao	QD	21	20	56	0·9952 ± 0·0165	0·023773 ± 0·012266	18·186643 ± 8·405941
Rudong	RD	24	19	51	0·9746 ± 0·0207	0·020815 ± 0·010725	15·923174 ± 7·359965
East China sea	ECS	48	35	67	0·9761 ± 0·0120	0·022969 ± 0·011525	17·571561 ± 7·943199
Taizhou	TZ	26	22	59	0·9877 ± 0·0139	0·023829 ± 0·012169	18·229236 ± 8·356229
Xiamen	XM	22	15	48	0·9264 ± 0·0457	0·018056 ± 0·009404	13·812867 ± 6·447948
South China sea	SCS	83	71	98	0·9947 ± 0·0032	0·023931 ± 0·011879	18·307231 ± 8·201301
Huizhou	HZ	9	9	17	1·0000 ± 0·0524	0·007047 ± 0·004254	5·391000 ± 2·868264
Zhanjiang	ZJ	27	24	61	0·9915 ± 0·0125	0·023838 ± 0·012154	18·236378 ± 8·348846
Beihai	BH	28	27	62	0·9974 ± 0·0104	0·024730 ± 0·012573	18·918585 ± 8·639238
Sanya	SY	19	18	47	0·9942 ± 0·0193	0·011609 ± 0·006258	8·880851 ± 4·283715
Total samples	Total	224	164	134	0·9877 ± 0·0032	0·023422 ± 0·011546	17·917672 ± 7·982752

N, sample size; H, number of haplotypes; S, number of segregating sites; h, haplotype diversity (±S.D.); $π$, nucleotide diversity (±S.D.); k, mean pairwise difference (±S.D.).

polymorphic sites (of which 84 were parsimony informative) with 191 transitions, 27 transversions and no indels. Sequence comparisons revealed 164 haplotypes in 224 individuals, most of which are unique, but with 17 that are shared among different local populations. Sequence divergence (Tamura and Nei distance) among haplotypes ranged from 0·1 to 5·6%, with an average of 2·4%. The nucleotide composition of the fragment was also characterized as A + T-rich (A: 24·87%, T: 38·67%). The overall diversities of haplotype and nucleotide were 0·9877 and 0·023422 respectively, indicating a high level of haplotype diversity and low nucleotide diversity (Table 2).

Phylogenetic analysis

Based on the sequences of the partial COI gene of *P. minor*, the NJ tree reconstructed using Tamura and Nei model with the gamma distribution 0·6798 was composed of two clades with high bootstrap support values (Fig. 2). Haplotypes from all 10 sample localities along the coast of China were irregularly scattered in these two clades. Except for a few differences in some small branches, the topology of the BI tree was very similar to that of the NJ tree presented here, and the posterior probabilities of the BI tree were also provided for the main clades of NJ tree (Fig. 2). Likewise, the network analysis also revealed two clusters with star-like patterns, and the haplotypes in the two clusters correspond to those in the two clades presented in the NJ tree (Fig. 3). Net average genetic distance (Tamura and Nei with gamma correction) between the two clades was 2·1%. Utilizing the mutation rate of 10% per million years (Li *et al.* 2011), it can be estimated that the separation occurred approximately 105 000 years ago, during the late Pleistocene era.

Similarly, phylogenetic analyses of *K. scombri* revealed two distinct lineages, which were reflected on the NJ tree, the BI tree and also the haplotype network (Figs 4 and 5). Both lineages include individuals from all sampling locations along the coast of China, i.e. they do not appear to have geographic structure. In the network analysis, each cluster exhibited a star-like pattern with large numbers of unique haplotypes surrounding some dominant haplotypes such as H13, H14 and H18, which is the signature of populations with a history of expansion (Avise, 2004). Based on the net average genetic distance between the two lineages (3·3%), their divergence is estimated to date back to approximately 165 000 years ago (in the middle Pleistocene).

Population structure

The pairwise F_{ST} values (Table 3) did not significantly differ ($P > 0·05$) among populations of *P. minor* along the coast of China, which implied non-significant genetic structure existed in the range investigated. This result was further supported by hierarchical AMOVA tests which suggested that the genetic variation within populations accounted for 100·59%, while those among the three marginal seas and among populations in each of these marginal seas were all negative (Table 4). Furthermore, the exact test of population differentiation (non-differentiation exact P values) also showed non-significant differences among the 10 populations (Table 3).

As for *K. scombri*, significant F_{ST} values were detected between SY and all other sample sites, between HZ and the other six localities, and the six comparisons within YS and ECS (Table 5), which suggests that the population of *K. scombri* has undergone genetic differentiation along the coast of China. AMOVA revealed significant genetic differences at two hierarchical levels: 14·78% ($P = 0·00$) among populations within the three marginal seas and 89·93% ($P = 0·00$) within populations (Table 4).
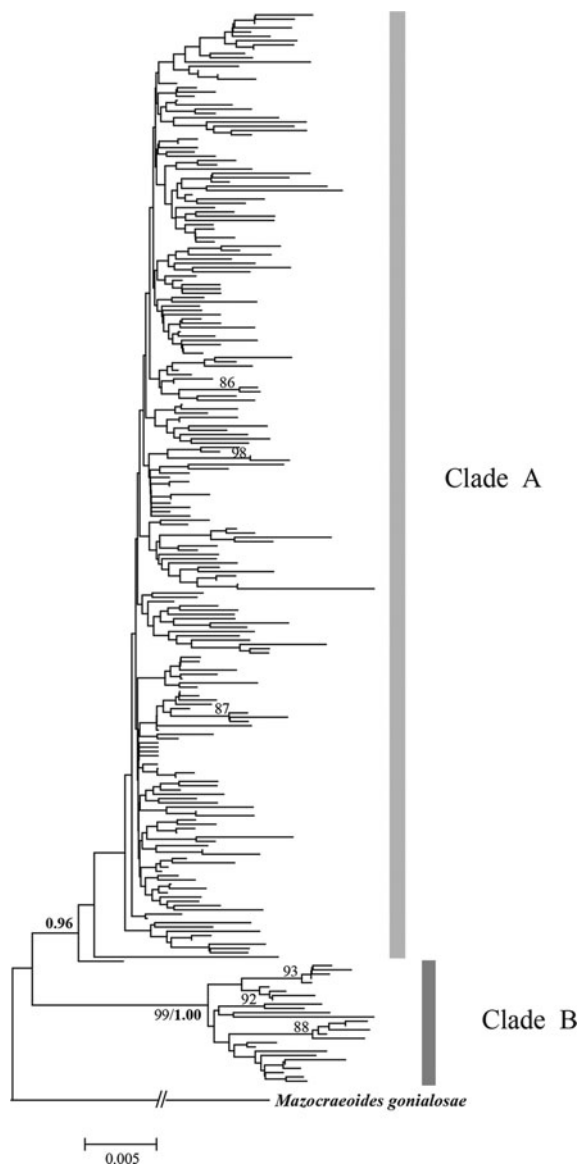
Fig. 2 Neighbor-joining tree for cytochrome c oxidase subunit 1 (COI) gene haplotypes of *Pseudokuhnia minor*. Bootstrap supports of >80% are shown at nodes. Bayesian posterior probabilities (1000 replications) are provided for main clades in a bold font.

The exact test of population differentiation did not detect significant differences among the 10 populations except in the comparisons of XM–RD, XM–TZ, XM–SY and SY–RD (Table 5).

Historical demography

The mismatch distributions for clades A and B of *P. minor* were clearly unimodal (Fig. 6), closely matching the expected distributions under the sudden expansion model. Moreover, the goodness of fit test showed that no mismatch distributions for these two clades deviated significantly ($P > 0.05$) from predicted values under the sudden expansion model of Rogers and Harpending (1992) (Table 6). The results of the two neutrality tests are consistent with the mismatch

analyses. The Fs values of the two clades were negative and highly significant ($P < 0.01$). Tajima's D values were negative and significant for clade A ($P = 0.0001$), and nearly significant for clade B ($P = 0.0567$) (Table 6). Collectively, this body of evidence suggests that *P. minor* along the coast of China experienced population expansion in the past. Based on the observed values of the age expansion parameters (τ), 8·49 for clade A and 8·31 for clade B (Table 6), and the mutation rate of 10% per million years mentioned above, it is estimated that the past population expansion started approximately 28 000 and 27 000 years ago for these two clades, respectively.

Both lineage A and lineage B of *K. scombri* showed a unimodal mismatch distribution, the model for populations having experienced sudden past expansion (Fig. 6). The P_{SSD} and raggedness tests could not reject the expansion hypothesis. Consistently, Fu's Fs and Tajima's D tests for both lineage A and B were negative and highly significant ($P < 0.01$) (Table 6), also providing evidence of population expansion. Likewise, it was estimated that the population expansion of the two lineages of *K. scombri* occurred about 11 000 and 16 000 years respectively, based on the τ values of lineages A and B.

DISCUSSION

Historical demography and genetic diversity

The large glacial–interglacial cycles induced by climate oscillations in the late Quaternary period (the past one million years) are considered to have caused a series of oceanographical alterations of sea levels, current patterns and many coastal habitats (Bond *et al*. 1997). During the Last Glacial Maximum (LGM) of the Pleistocene Period, sea levels were estimated to be approximately 120–140 m lower than their current level (Lambeck *et al*. 2002). In that period, the entire Bohai Gulf and the YS were exposed (Fig. 1), the ECS was reduced into an elongated trough (Wang and Sun, 1994), and the SCS became a semi-enclosed gulf connected with the Pacific Ocean mainly through the Bashi Strait (Wang, 1999). These changes may have contributed to the demographic decline, distribution isolation, and even extinction of many coastal marine organisms. For the two species of monogeneans, *P. minor* and *K. scombri*, on the host, *S. japonicus*, along the coast of China, both the mismatch distribution analysis and the neutrality tests suggested a history that included population expansion. This conclusion was also supported by the P_{SSD} and raggedness tests, and the star-like median networks that they revealed. In spite of different estimations of expansion time for species *P. minor* and *K. scombri* (28 000–27 000 and 16 000–11 000 years ago, respectively), both fell in the late Pleistocene era (126 000–10 000 years ago). These two host-
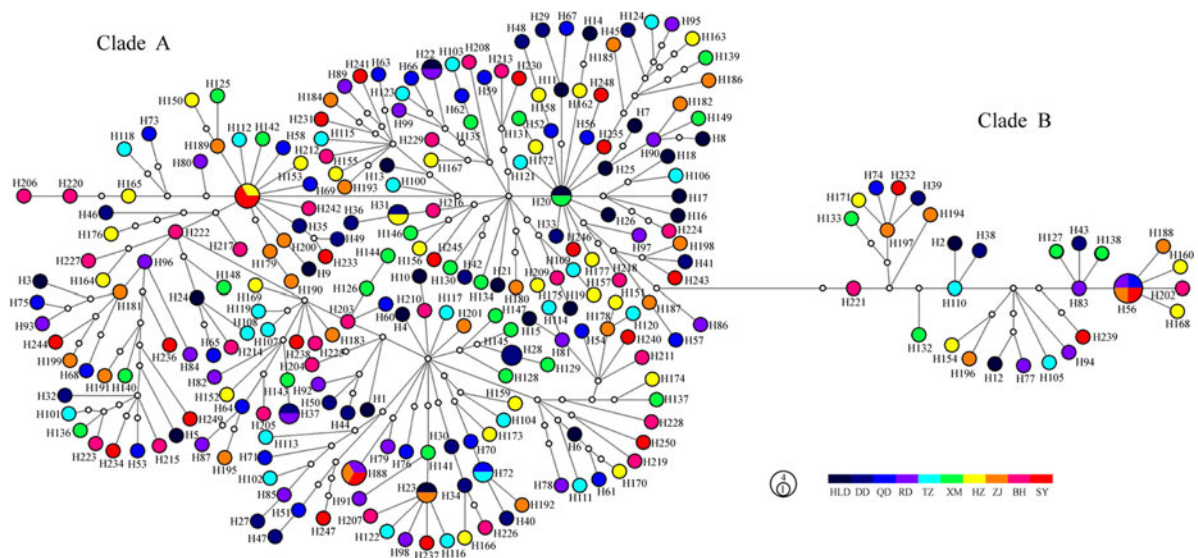
Fig. 3 Median-joining network for COI haplotypes of *Pseudokuhnia minor*. The sizes of the circles are proportional to haplotype frequency and the colours represent the population (HLD, Huludao; DD, Dandong; QD, Qingdao; RD, Rudong; TZ, Taizhou; XM, Xiamen; HZ, Huizhou; ZJ, Zhanjiang; BH, Beihai; SY, Sanya) to which they belong; small hollow circles represent missing haplotypes.
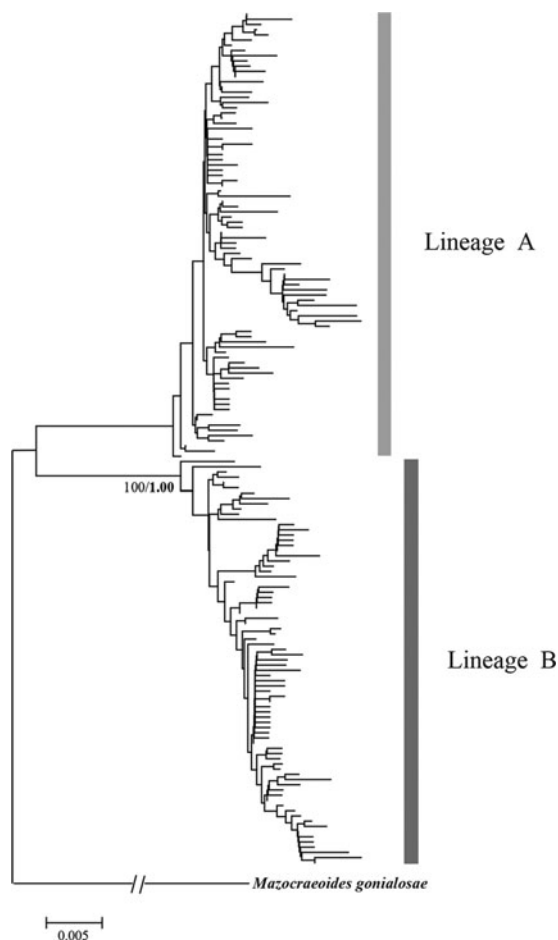

Fig. 4 NJ tree for COI haplotypes of *Kuhnia scombri*. Bootstrap supports of >80% are shown at nodes. Bayesian posterior probabilities (1000 replications) are provided for main clades in a bold font.

specific parasites, *P. minor* and *K. scombri*, might have also suffered population decline and isolation due to the decline of their host population during the LGM. Afterwards, with the glacial retreat and the sea level rise, the populations of these two species of monogeneans increased in both quantity and distribution with the dispersal of their hosts from different refuges. Previous studies on the phylogeography of monogeneans, *M. gonialosae* and *Gotocotyla sawara* along the coast of China also suggested the existence of historic population expansion in the Pleistocene era (Li *et al.* 2011; Shi *et al.* 2014).

Both *P. minor* and *K. scombri* showed high haplotype diversities and low levels of nucleotide diversity, indicating the existence of population expansion in the history, possibly caused by bottle-neck or founder events (Grant and Bowen, 1998). In general, haplotype diversity close to 1 is no longer considered to be an informative measure of polymorphism, and is not useful in the discrimination of different populations (Li, 1997). On account of high haplotype diversities of both species (close to 1), nucleotide diversity was used to compare their genetic diversities. The much lower nucleotide diversity of *P. minor* population than that of *K. scombri* could be attributed to either a lower rate of evolution or younger age of *P. minor*, both of which would result in the accumulation of less diversity (Glennon *et al.* 2008).

Phylogeographic congruence

For both species of parasites, phylogenetic analyses constructed a tree composed of two clades of no
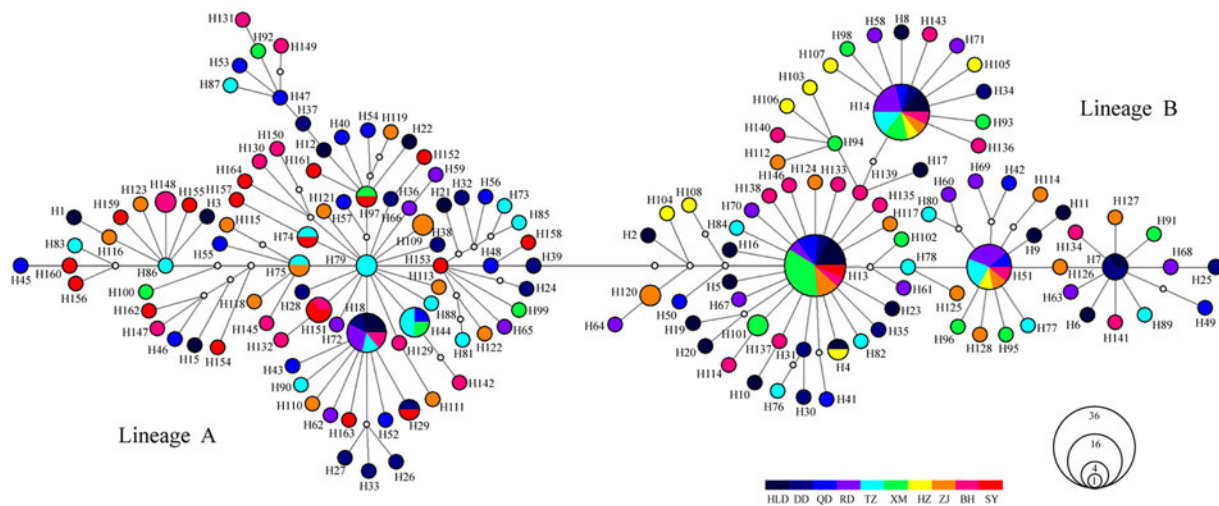
Fig. 5 MJN for COI haplotypes of *Kuhnia scombri*. The sizes of the circles are proportional to haplotype frequency and the colours represent the population (HLD, Huludao; DD, Dandong; QD, Qingdao; RD, Rudong; TZ, Taizhou; XM, Xiamen; HZ, Huizhou; ZJ, Zhanjiang; BH, Beihai; SY, Sanya) to which they belong; small hollow circles represent missing haplotypes.

geographic association along the coast of China, which is characteristic of reblending populations following previous isolation. The divergence of the two clades of both *P. minor* and *K. scombri* occurred during the Pleistocene era. As mentioned above, the populations of both species might have been isolated in different glacial refuges with the decline of sea level during the glacial period, with a subsequent remixing with the rise of sea level during the postglacial period. Similarly, the population of the host of these two parasites, *S. japonicus* in the coast of China, was also deduced to have experienced population isolation and remixing during the Pleistocene era based on the topological structure of two lineages on the NJ tree (Yan *et al*. 2015). This coherence between host and parasites revealed in our study agrees with the statement that parasites with a high degree of host specificity are expected to have consistent genealogy with their host (Beebee and Rowe, 2008). It is therefore infer that the phylogeographic patterns of monogeneans are highly influenced by their respective hosts.

Comparative genetic structure

Marine organisms generally show low levels of genetic differentiation and high levels of gene flow across wide geographic regions due to the high dispersal potential of planktonic eggs, larvae and adults, and the absence of physical barriers between ocean basins or adjacent continental margins (Hewitt, 2000). In the present study, *P. minor* conforms to this pattern, suggesting that this population is in a state of a panmixia, with a high rate of gene exchange among different local populations along the coast of China. For parasitic organisms, population genetic structure is determined

not only by the dispersal abilities of parasites themselves, but is also profoundly influenced by the biological characteristics of their hosts (Miura *et al*. 2006). As for the host in the present study, chub mackerel (*S. japonicus*) is a pelagic, migratory and large-shoal-forming species (Lockwood, 1988), which is widespread in the warm and temperate transition coastal areas and the adjacent seas of the Atlantic, Pacific and northwest Indian oceans (Collette and Nauen, 1983). Since monogenean parasites cannot parasitize the pelagic eggs and larvae of fish (Cribb *et al*. 2000), their primary dispersal mechanism is by way of adult fish. Thus, the seasonal long distance migration of adult *S. japonicus* might also facilitate the gene flow of its parasites. The passive drift of eggs and larvae of parasites with sea currents may also promote the mixing of parasites among the YS, ECS and SCS although the monogenean larvae, oncomiracidia, can normally swim and survive for a very limited time before they attach to a suitable host (Gannicott and Tinsley, 1998). In addition, historical geological processes have had profound impacts on the population structure of marine organisms (Glennon *et al*. 2008). The insufficient time to attain migration-drift equilibrium after a recent range expansion might be associated with the high genetic similarity within the region studied (Slatkin, 1993). Similar explanations have also been proposed in the studies of monogeneans, *M. gonialosae* and *G. sawara* along the coast of China (Li *et al*. 2011; Shi *et al*. 2014).

Different from *P. minor*, *K. scombri* on the same host and sampled in the same locations as *P. minor* showed genetic differentiation, which was supported by both the AMOVA test and analyses of pairwise F_{ST} values. Sympatric marine organisms with

Table 3. Pairwise F_{ST} (below diagonal) and P values for exact test of population differentiation (above diagonal) among populations of *Pseudokuhnia minor*

	HLD	DD	QD	RD	TZ	XM	HZ	ZJ	BH	SY
HLD		0·48926	1·00000	1·00000	1·00000	1·00000	1·00000	1·00000	1·00000	0·50082
DD	−0·00339		0·49653	0·71878	0·48450	0·45681	0·75307	0·47350	0·48590	0·24675
QD	−0·01515	−0·01200		1·00000	1·00000	1·00000	1·00000	1·00000	1·00000	0·74578
RD	0·00247	−0·00721	−0·00237		1·00000	1·00000	1·00000	1·00000	1·00000	0·85849
TZ	−0·01453	−0·00599	−0·01175	−0·00268		1·00000	1·00000	1·00000	1·00000	0·49867
XM	0·00644	−0·01039	0·00524	−0·00704	0·00278		1·00000	1·00000	1·00000	0·50314
HZ	−0·00455	−0·01297	−0·00689	−0·00481	−0·00892	−0·01135		1·00000	1·00000	1·00000
ZJ	0·01067	−0·00329	0·00501	−0·01146	0·00212	−0·01274	−0·01066		1·00000	0·87499
BH	−0·00355	−0·00829	−0·01483	0·00165	−0·00590	0·00298	0·00038	0·01192		0·50715
SY	−0·00825	−0·01120	−0·01805	−0·00478	−0·00938	−0·00394	−0·00741	−0·01114	−0·00944	

HLD, Huludao; DD, Dandong; QD, Qingdao; RD, Rudong; TZ, Taizhou; XM, Xiamen; HZ, Huizhou; ZJ, Zhanjiang; BH, Beihai; SY, Sanya.

Table 4. Pairwise F_{ST} (below diagonal) and P values for exact test of population differentiation (above diagonal) among populations of *Kuhnia scombri*

|	HLD	DD	QD	RD	TZ	XM	HZ	ZJ	BH	SY
HLD		0·83188	0·76253	0·47102	0·14881	0·37080	0·61328	0·35999	0·65168	0·16677
DD	0·07971		0·79822	0·29736	0·26997	0·05997	0·81718	0·38179	0·80113	0·66256
QD	0·12152*	−0·02954		0·47058	0·57337	0·41668	0·92362	0·72591	0·89644	0·58355
RD	−0·02817	0·06774	0·11394*		0·62406	0·02870*	0·63819	0·28619	0·61775	0·03613*
TZ	0·09346*	−0·03337	−0·03491	0·08314		0·01242*	0·73390	0·22029	0·47673	0·11924
XM	−0·02725	0·13059*	0·17645**	−0·02494	0·14186*		0·07520	0·10182	0·06238	0·04474*
HZ	0·10195	0·33468**	0·38540**	0·10605	0·34346**	0·06575		0·70951	0·95590	0·54747
ZJ	0·02645	−0·01935	−0·00511	0·02066	−0·01464	0·06310	0·24912**		0·55850	0·23493
BH	0·01849	−0·01732	0·00017	0·01331	−0·01131	0·04987	0·22929*	−0·02794		0·81673
SY	0·44466**	0·18839**	0·12726*	0·44285**	0·16128*	0·51917**	0·73898**	0·25607**	0·25940**	

HLD, Huludao; DD, Dandong; QD, Qingdao; RD, Rudong; TZ, Taizhou; XM, Xiamen; HZ, Huizhou; ZJ, Zhanjiang; BH, Beihai; SY, Sanya.
* $P < 0.05$.
** $P < 0.01$.

Table 5. *Summary of hierarchical analysis of molecular variances for Pseudokuhnia minor and Kuhnia scombri*

	Source of variation	Variance components	Percentage of variation	F statistics	P value
Pseudokuhnia minor	Among groups	−0·01152	−0·19	$F_{CT} = -0.00188$	0·78552
	Among populations within groups	−0·02460	−0·40	$F_{SC} = -0.00402$	0·73175
	Within populations	6·14894	100·59	$F_{ST} = -0.00591$	0·85040
Kuhnia scombri	Among groups	−0·42161	−4·71	$F_{CT} = -0.04714$	0·78968
	Among populations within groups	1·32233	14·78	$F_{SC} = 0.14118$	0·00000
	Within populations	8·04384	89·93	$F_{ST} = 0.10070$	0·00000
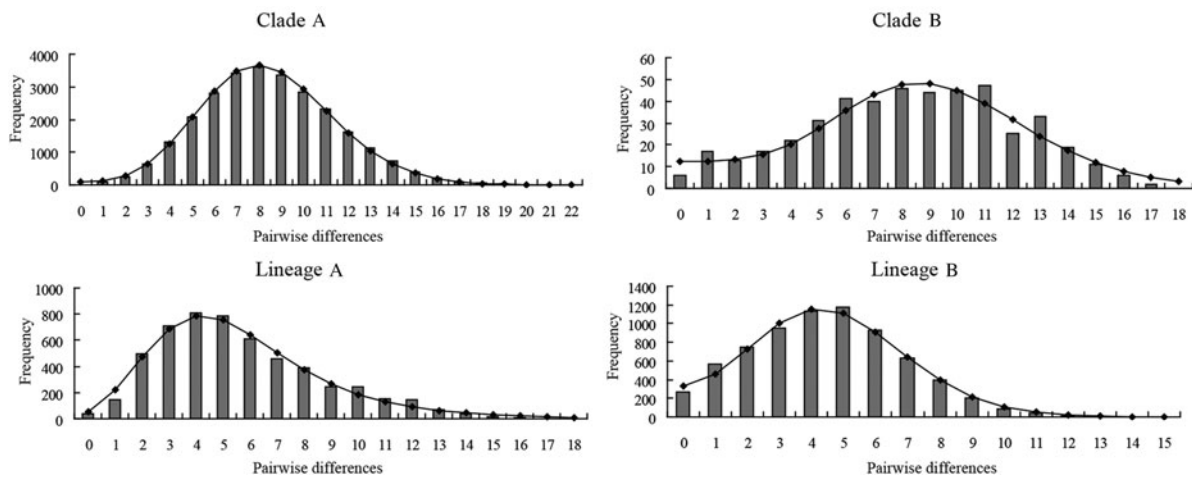
Fig. 6 Mismatch distribution of COI haplotypes for the two clades (Clades A and B) of *Pseudokuhnia minor* and two lineages (lineages A and B) of *Kuhnia scombri*. The observed pairwise differences are shown as bars and the expected values under the sudden expansion model are solid lines.

Table 6. *Tajima's D, Fu's F_S statistics, corresponding P values and mismatch distribution parameter estimates for each clade of Pseudokuhnia minor and Kuhnia scombri based on cytochrome c oxidase I sequence data*

	Tajima's D		Fu's F_S			Goodness-of-fit tests			
	D	P	Fs	P	τ	SSD	P	HRI	P
Pseudokuhnia minor									
Clade A	−2·29319	0·00010	−24·31953	0·00050	8·48633	0·00009	0·85270	0·00523	0·78540
Clade B	−1·42148	0·05670	−19·01984	0·00000	8·30664	0·00160	0·94730	0·00582	0·96230
Kuhnia scombri									
Lineage A	−2·22186	0·00070	−25·25663	0·00000	3·42578	0·00071	0·64570	0·01005	0·56680
Lineage B	−2·26066	0·00040	−25·63164	0·00000	4·94531	0·00043	0·94870	0·00892	0·89190

different population genetic structures were also revealed in fishes *S. scombrus* and *S. japonicus* in the Mediterranean Sea (Zardoya *et al.* 2004), *Lateolabrax japonicus* and *Lateolabrax maculatus* in the Northwestern Pacific Ocean (Liu *et al.* 2006), and in monogeneans *Haliotrema aurigae* and *Euryhaliotrematoides grandis* in the South Pacific Ocean (Plaisance *et al.* 2007). Observed differences were ascribed to their specific evolutionary and biological features, such as life histories and dispersal strategies (Plaisance *et al.* 2007). In this study, the speculation that *P. minor* has lower evolution rate or that *K. scombri* being an older species could help to account for their different population genetic structures. Although the biology of *K. scombri* has not yet been studied due to the difficulties in culturing captive hosts, a more abundant population of *P. minor* than that of *K. scombri* (unpublished data) was observed, which may imply that the population of the former has had fewer opportunities to

accumulate genetic structure locally. In addition, genetic divergence and even speciation could be resulted from host switches in the evolutionary history of parasites. There exists an alternative host of these two monogeneans, *Scomber australasicus*, along the coast of China. However, the distributional overlap of these two species of hosts would have largely abated the role of host switch to the genetic structure of these two parasites.

We acknowledge that the main conclusions of the present study rely on information from a single mitochondrial gene. Further studies on the biology of these two monogeneans and population genetic structure in a larger geographical range with more mitochondrial and/or nuclear markers are needed for an improved understanding of the geographical pattern of these parasites.

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