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ORIGINAL PAPER

Pathways of cryptic invasion in a fish parasite traced using coalescent analysis and epidemiological survey

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Abstract Introduced species have the potential to outperform natives via the introduction of new parasites to which the native ecosystem is vulnerable. Cryptic diversity within an invasive species can obscure invasion patterns and confound proper management measures. The aim of this study is to use coalescent theory based methodology to trace recent routes of invasion in populations of *Ligula intestinalis*, a globally distributed fish parasite possessing both native and recently introduced populations in North Africa. Molecular analyses of mitochondrial DNA discerned a pronounced genetic divergence between introduced and native populations. Distribution of mitochondrial haplotypes demonstrated common origin of European populations with North African parasites sampled from introduced fish species in Tunisia. To test the suggested pathway of introduction, microsatellite data were examined in a model-based coalescent analysis using the software MIGRATE, where Europe to Tunisia direction of migration was favoured over alternative hypotheses of gene flow. Specificity of Tunisian populations to different host species was assessed in an epidemiologic survey confirming prevailing host-based division between introduced and native parasites in North Africa. This approach combining advanced analysis

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of molecular markers with host-specificity data allows revealing the evolution of host-parasite interactions following biological invasion and provides basis for devising future management measurements.

Keywords Aquaculture · Coevolution · Directionality of migration · Population split · *Ligula intestinalis* · Parasite introduction

Introduction

Species introductions and invasions represent an important threat to the functioning of ecosystems (Clavero and García-Berthou 2005), affect biodiversity in invaded areas and may lead to significant economic loss (Pimentel et al. 2005). Unlike free-living organisms, which are often introduced to new areas deliberately, parasites are usually inserted unintentionally, simultaneously with their hosts. Despite their partially hidden engagement, parasites often play a key role in the invasion process of their hosts (Prenter et al. 2004). When transferred to native species, invading parasites can rapidly cause species loss in favor of the introduced host species. Examples of introduced parasites having detrimental impact on local populations include malaria in Hawaii birds (Van Riper III et al. 1986), poxvirus in red squirrels (Rushton et al. 2006) and nematodes in European eel (Sasal et al. 2008; Wielgoss et al. 2008).

Despite numerous examples of detrimental impact of the introduced parasites or pathogens on terrestrial and aquatic ecosystems, very few studies explored the population genetic parameters of introduced parasitic species or traced the routes of invasion using molecular data. Surprisingly, amongst the few cases where the relationships between native and introduced populations of parasites were studied, it was often found that introduced populations may represent several genetically isolated clusters with independent origin such as the giant river fluke parasitizing deer (Králová-Hromadová et al. 2011). Using mitochondrial (mtDNA) haplotype data, it was demonstrated that at least two independent introductions of flukes from North America to Europe occurred, each from a different area of the original range. In another case, several lineages of a parasite differing in their host preference were found in the newly invaded area. Chytrid fungus that is rapidly spreading in amphibian populations world-wide was shown to comprise several lineages with different affinity and pathogenicity on different amphibian hosts occurring in wild or bred in captivity in Japan (Goka et al. 2009).

To devise effective measures against future introduction of new species and to prevent recurrent introduction of already established invaders, it is important to identify the invasion routes and the directionality of gene flow, especially in cases where historical information is lacking or the biological invasion emerges and propagates very quickly without prior notice (Mergeay et al. 2006; Dlugosch and Parker 2008). Estimating the direction and intensity of gene flow in the situation where the exact source population is unknown may be confounded by recurrent introductions and short time since the initial colonization of new areas (Therriault et al. 2005). Strong genetic links may prevail for generations in populations that were only recently separated from their ancestors, especially in cases where new area was colonized by a large sample of the original population (Wattier et al. 2007).

Frequently, the routes of species introduction are situated between the new and old world ecosystems of the temperate and tropic zones (e.g. Brown and Stepien 2010; Ascunce et al. 2011). Here, we explore a situation in which a fish stock of European origin has been introduced into several freshwater systems of North Africa. In an attempt to enrich the local fish fauna, and rehabilitate dam reservoirs of Tunisia, various European fish species (particularly cyprinids) were introduced and stocked to complement the sole indigenous species, the barbel *Barbus callensis*, and the minnow *Pseudophoxinus callensis*. The introductions took place in 1960s following the Tuniso-German cooperation project GTZ (Losse et al. 1991). For instance, the roach (*Rutilus rubilio*) and the rudd (*Scardinius erythrophthalmus*) were introduced from southern Europe (primarilly Italy) to serve as forage for fish of economical importance, especially for the sander *Stizostedion lucioperca* (Kraïem 1991; Losse et al. 1991).

These introduced species are known to be potential hosts for the diphyllobothriidean cestode *Ligula intestinalis* (e.g. Manilla et al. 1984). The parasite possesses a three-host lifecycle with copepods and fish as intermediate hosts and piscivorous birds as the definite host (Dubinina 1980). The secondary larvae (plerocercoids) inhabiting body cavities of cyprinid

fish cause severe pathogenic effects on the fish growth (Loot et al. 2002), morphology (Loot et al. 2001a), sexual development (Carter et al. 2005) and behaviour (Brown et al. 2001), leading to high mortality rates in fish populations (Loot et al. 2001b). Due to its importance in fish aquaculture and popularity in ecological and evolutionary studies *L. intestinalis*, has become a favourite parasitic model organism (Hoole et al. 2010) and its host preferences and geographical distribution in Euro-Asia are very well explored (Dubinina 1980). In Tunisia, the presence of the parasite was reported in the introduced roach *R. rubilio* and rudd *S. erythrophtalamus* in Sidi Salem and Nebhana dam reservoirs (Kraïem 1991; Djemali 2005; Bahri-Sfar et al. 2010). Elsewhere in Africa, *Ligula* infection has only been reported from native cyprinid species (Khalil and Polling 1971; Dejen et al. 2006).

Earlier studies dealing with genetic variability of *Ligula* populations on a global scale showed that *L. intestinalis* comprises several genetically isolated lineages with separated host spectra and distinct geographic distribution (Bouzid et al. 2008a, b; Štefka et al. 2009). The populations inhabiting European and North African regions were found to comprise two sympatric mitochondrial clades, termed clade A and B, which markedly differed in their host preference. The clade A was suggested to have been introduced to the North African area with its cyprinid hosts, whereas clade B was found to be native in both areas. Using microsatellite data, the study of Štefka et al. (2009) found significant amount of structure between the introduced and native populations of clade A, whereas no indication of population structure was found inside the European continent despite geographically and host extensive sampling. The uniformity of the European clade A populations was accounted to the dispersion with bird hosts mediating extensive gene flow. Due to short duration of the infection in the definitive host, it was suggested that Mediterranean Sea represents a barrier impassable for parasites with their bird hosts migrating across the sea annually, however the directionality of the ancestral genetic connection between Europe and North-Africa and the potential of introduced parasites to threat local fish fauna were left unexplored.

Using molecular and epidemiologic data we aim to (1) consolidate the phylogenetic position of native and introduced populations of *L. intestinalis* in the Euro-Mediterranean area with respect to the global distribution of the species; (2) explore differences in host preference between native and introduced populations in North Africa and (3) investigate the directionality of gene flow between *L. intestinalis* populations in Europe and the introduced North African populations.

Materials and methods

Study area and fish sampling

Fish specimens from introduced European fish, roach (*R. rubilio*) and rudd (*S. erythrophthalmus*) were sampled in Tunisia from summer 2004 to autumn 2005. The sampling of these species was realized in Sidi Salem reservoir, which constitutes the largest reservoir of drinking water in the north-west of Tunisia (surface of 4,300 ha and depth of about 10 m at normal level) and Nebhana reservoir located in central Tunisia (surface of 540 ha and depth of about 10 m at normal level). In order to prevent young fish that are not infected with plerocercoids from capturing, net meshes sized 40 mm were used. In the same period, native barbels (*B. callensis*) were sampled in Sidi Salem and Nebhana sites using seine-net. Collected parasites were stored in 70 % ethanol and kept in freezer prior to molecular analyses.

Parasite specimens from native minnow (*P. callensis*) were provided by Dr. M. Kraïem and collaborators from the National Institute of Marine Sciences and Technologies, Salammbô, Tunisia. These specimens were collected in 2004 using the seine-net throughout the banks of Joumine (surface of 234 km^2 with a depth of about 1 m) and Remel (surface of 684 Km^2, with a depth of about 1 m 30) (North and North east of Tunisia respectively) where a great number of *P. callensis* occur (Kraïem 1983). *P. callensis* parasite specimens were preserved in denatured ethanol since their collection in 2004.

Parasite samples from Algeria provided by Algerian colleagues (see Acknowledgement) were originally fixed in formalin and later transfered to pure ethanol in our laboratory. Samples from the European area of distribution were collected in the frame of previous studies (Bouzid et al. 2008b; Štefka et al. 2009) (see map in Fig. 1 for localities sampled in the

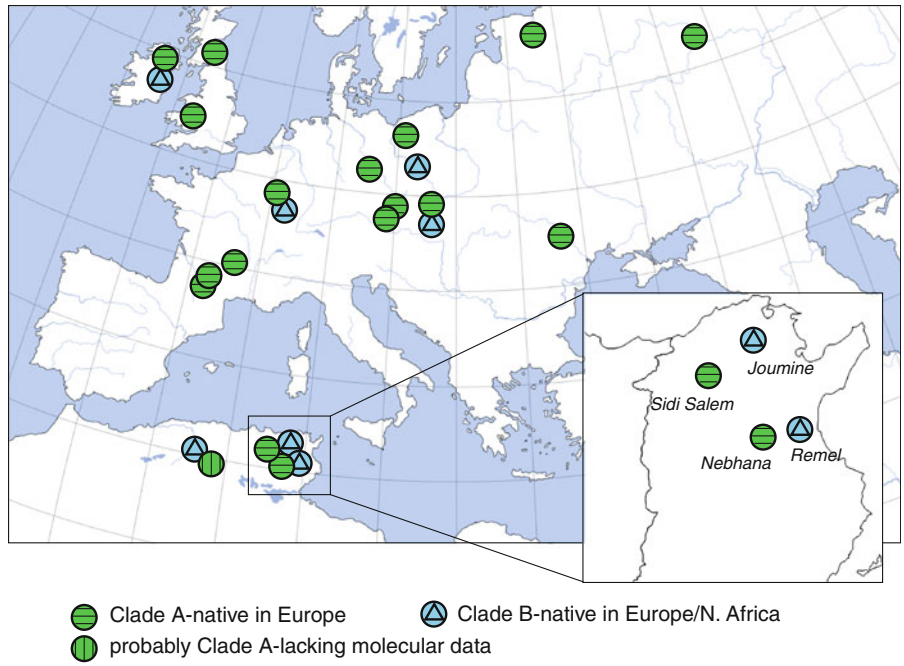


Fig. 1 Map of the distribution of *L. intestinalis* populations studied in the Euro-Mediterranean area of distribution. Detailed information on localities and sample sizes is provided in Table 1

European and north African areas of distribution). Complete list of localities and specimens analysed in this study is available in Table 1.

Parasite analyses

For material collected in Tunisia, each individual fish was dissected to count plerocercoids present in the abdominal cavity. Plerocercoid larvae of *L. intestinalis* were identified using the determination key of Dubinina (1980). The Prevalence (P) and Mean Intensity (MI) were calculated as defined by Margolis et al. (1982). No epidemiology data were available for samples from Algeria, which were collected prior to this study.

PCR amplification and DNA sequencing

Molecular characterization of collected parasites were carried out using concatenated matrix of sequences of cytochrome oxidase I (COI) and cytochrome b (COB) genes, and then compared to available sequences from Genbank obtained earlier (Bouzid et al. 2008b) belonging to specimens from a large geographic scale. Details of collection localities, fish host species, number of specimens analysed and their accession numbers are given in Table 1.

Total genomic DNA was extracted using the Promega DNA isolation kit (Promega, Madison, WI) from samples stored in ethanol. PCR reactions were performed using conditions and primers from Bouzid et al. (2008a, b). Purified DNA (20 ng/µl) was sequenced directly with ABI BigDye chemistry using the same primers as for DNA amplification.

DNA extractions using Chelex 100 Resin (Sigma-Aldrich) were applied to Algerian samples fixed in formalin. Approximately 2 mm of dried plerocercoid tissue was placed into a tube containing 100 µl of 10 % Chelex solution. Tubes were kept at 95 °C for 30 min and vortexed occassionally. Prior to PCR, the tubes were vortexed and spinned down on a microcentrifuge and 2 µl of supernatant were used for amplification. Since some extractions failed to amplify, probably due to DNA degradation caused by formalin, new sets of primers for COI and COB were designed (Table 2). These internal primers annealed in conserved regions inside the two genes and were used with regular forward and reverse PCR primers in the PCRs as described

Table 1 Geographic origin and host species of *Ligula* samples

Country of origin	Collection locality	Host species	Symbol	Number of samples analysed for both genes (haplotype numbers used in Figs. 1, 2)	GenBank accession number	
					COB	COI
Algeria	Keddara reservoir	*Barbus* sp.	ALG1Bsp	4 (1, 2, 4)	JQ279107/44-45/48	JQ279072
	Taksebt reservoir	*Barbus setivimensis*	ALG2Bs	3 (2, 3)	JQ279108/46-47	JQ279070-71/73
	Oued Hamiz	*Barbus* sp.	ALG3Bsp	10 (1, 4, 5, 7, 8, 9)	JQ279106/09-12/14-15 [EU241143-45]	JQ279068/74-79 [EU241219-21]
	Oubeira reservoir	*P. callensis*	ALG4Pc	1 (6)	JQ279113	*
Australia	Goodga river	*Galaxias truttaceus* (Osmeriformes)	AU Gt	1 (10)	[EU241146]	[EU241222]
	Moates lake	*Galaxias maculatus* (Osmeriformes)	AU Gm	1 (11)	[EU241147]	[EU241223]
Canada	Dalpec lake	*Coulsius plumbeus*	CA Cp	1 (14)	[EU241152]	[EU241228]
	Dumbo lake	*Semotilus atromaculatus*	CA Sa	4 (12, 13, 15, 16)	[EU241148-49/50-51]	[EU241224-27]
China	Dong Tink lake	*Hemiculter bleekeri*	CN Hb	2 (18)	[EU241153-54]	[EU241229-30]
	Zhanghe reservoir	*Neosalanx taihuensis* (Osmeriformes)	CN Nt	3 (17, 19)	[EU241155-57]	[EU241232/34/36]
Czech Republic	Lipno reservoir	*Rutilus rutilus*	CZ1Rr	4 (22, 26, 35, 36)	[EU241159/65/69/70]	[EU2412477-81]
	Zelivka reservoir		CZ2Rr	1 (37)	[EU241178]	[EU241282]
		Alburnus alburnus	CZ2Aa	1 (34)	[EU241177]	[EU241246]
	Nové Mlýny reservoir	*Abramis brama*	CZ3Ab	5 (27, 28, 29, 30, 31)	[EU241166/79/80/82/84]	[EU241263-70/83-86]
	Záhlinice and Tlumačov ponds	*Podiceps cristatus*—bird host	CZ4Pc	6 (20, 21, 23, 32)	[EU241164/67/68/74/87/90/91]	[EU241244-45/62/87/93]
		Mergus merganser—bird host	CZ4 Mm	1 (24)	[EU241193]	[EU241239]
	Tovacov pond	*P. cristatus*—bird host	CZ5Pc	1 (33)	[EU241186]	[EU241288]
Estonia	Peipsi lake	*A. brama*	EE Ab	5 (24, 43, 44, 45, 46)	JQ279121-22 [EU241160/92/95]	JQ279085-86 [EU241275-76/94]
Ethiopia	Tana lake	*Barbus humilis*	ET Bh	1 (47)	[EU241197]	[EU241295]
		Barbus tsanensis	ET Bt	1 (48)	[EU241196]	[EU241296]
		Barbus intermedius	ET Bi	1 (49)	[EU241198]	[EU241297]
France	Pareloup and Vioulou lakes	*A. alburnus*	FR1Aa	3 (21, 52, 53)	JQ279123-24 [EU241163/99]	JQ279087-88 [EU241258/60]
		R. rutilus	FR1Rr	5 (21, 41, 50)	JQ279117/19/42 [E]	JQ279081/83-84/104
	Muret and Lavernose lakes		FR2Rr	2 (41, 51)	JQ279118/43	JQ279082/105
		Blicca bjoerkna	FR2Bb	1 (51)	[EU241201]	[EU241259]
	Créteil reservoir	*R. rutilus*	FR3Rr	5 (50, 54, 55, 56, 57, 58)	JQ279125-26 [EU241172/73/200]	JQ279089/90 [EU241261/99/300, EU636655]

Table 1 continued

Country of origin	Collection locality	Host species	Symbol	Number of samples analysed for both genes (haplotype numbers used in Figs. 1, 2)	GenBank accession number	
					COB	COI
Germany	Müggelsee	*R. rutilus*	DE Rr	6 (34, 38, 39, 40, 41, 42)	JQ279116/20 [EU24185/202-04]]	JQ279080/84 [EU241273-74/301/02]
Great Britain	Scotland, river Gryfe	*R. rutilus*	GB Rr	1 (59)	[EU241205]	[EU241303]
		P. phoxinus	GB Pp	1 (60)	[EU241175]	[EU241304]
	Wales, Aberystwyth	*P. phoxinus*	GB Pp	1 (44)	[EU241161]	[EU241247]
N. Ireland	Lough Neagh lake	*R. rutilus*	IE Rr	3 (41, 61, 64)	[EU241117/206/07]	[EU241248-50]
		Gobio gobio	IE Gg	3 (20, 62, 63)	[EU241188/89/208]	[EU241290/305]
Poland	Wloclawski reservoir	*Rhodeus amarus*	PL Ra	1 (25)	[EU241194]	[EU241289/92]
Russia	Khanka lake, Far East	*Hemiculter lucidus*	RU Hl	1 (65)	[EU241209]	[EU241311]
	Rybinsk reservoir	*A. brama*	RU Ab	5 (44, 66, 67, 68, 69)	[EU241158/210/11/12/13]	[EU241251-56/58/309-10]
Tunisia	Sidi Salem reservoir	*R. rubilio*	TN1Rb	8 (70, 71, 73, 81)	JQ279127-29 [EU241214-17]	JQ279091-92 [EU241271/312-15]
		S. erythrophthalmus	TN1Se	5 (21, 72, 73, 80)	JQ279130/32/34/41 [EU241162]	JQ279093/95/97/103 [EU241272]
		B. callensis	–	–	–	–
		P. callensis	–	–	–	–
	Nebhana reservoir	*R. rubilio*	TN2Rb	2 (74, 75)	JQ279135-36	JQ279098-99
		S. erythrophthalmus	TN2Se	2 (73)	JQ279131/33	JQ279094/96
		B. callensis	–	–	–	–
		P. callensis	–	–	–	–
	Oued Joumine	*P. callensis*	TN3Psc	2 (76, 77)	JQ279137/38	JQ279100-01
	Oued Remel	*P. callensis*	TN4Psc	2 (78, 79)	JQ279139/40	JQ279102
Ukraine	Dniester river	*Carassius carassius*	UA Cc	1 (83)	[EU241218]	[EU241238]
		R. rutilus	UA Rr	1 (84)	[EU241176]	[EU241317]
		A. alburnus	UA Aa	1 (82)	[EU241181]	[EU241316]
Totals				199 (84)		

Distribution of 84 haplotypes (referred to under their GenBank Accession numbers) is given

GenBank accession number between [] refer to sequence retrieved from Bouzid et al. (2008b)

*, Sequencing failed due to formalin caused DNA degradation

–, No *Ligula* specimen was found in the corresponding host/locality

Table 2 COI and COB internal primers used to sequence *L. intestinalis* from Algeria fixed in formalin

Gene	Sequence (5′ to 3′)	Direction	Gene position
COI	TTTAGTTCAGTTACTATGATTATTGGC	Sense	913–938
COI	GCCAATAATCATAGTAACTGAACTAAA	Antisense	938–913
COB	GCTGCTACTGTGTTAACTGCAATAG	Sense	397–421
COB	CTATTGCAGTTAACACAGTAGCAGC	Antisense	421–397

above. Thus, each gene was amplified and sequenced in two smaller fragments (approximately 200 bp in length). Same PCR approach was applied to some of the Tunisian samples stored in denatured ethanol, which also showed lower amplification success.

Concatenated alignments of mitochondrial COI and COB genes were created in BioEdit (Hall 1999) without use of manual corrections. Program Collapse 1.2 (Posada 2004) was used to retrieve individual haplotypes.

Phylogenetic analyses

The evolutionary history of samples was inferred from the matrix of concatenated COI and COB haplotypes using Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian inference (BI) methods. The software Mega v.4 (Tamura et al. 2007) was employed for the MP analysis using the Close-Neighbor-Interchange algorithm with search level 3 (Nei and Kumar 2000), where the initial trees were obtained with the random addition of sequences (10 replicates). Calculation of bootstrap consensus tree was inferred from 1,000 replicates.

ML analysis was performed in PhyML v. 3.0 (Guindon et al. 2010). The analysis was run using GTR + G model and the parameters of gamma distribution were estimated from the data. The model of molecular evolution of sequences was selected using Akaike Information Criterion in Modeltest (Posada and Crandall 1998). Bootstrap support was obtained by 1,000 replications.

BI reconstruction of phylogeny was performed in MrBayes 3.2.16 (Huelsenbeck and Ronquist 2001) using 10 million Markov Chain Monte Carlo (MCMC) replications and two independent runs (4 chains each). Based on the Akaike Information Criterion in MrModeltest 2.3 (Nylander 2004), the GTR + I + G model was the best supported model of molecular evolution. Convergence between parameter estimates and the effective sampling sizes were checked in Tracer 1.5 (Rambaut and Drummond 2005). Credibility of obtained topologies was checked using program AWTY (Nylander et al. 2008), where consistency between two independent runs and posterior probability trends of the identified clades were inspected across successive MCMC steps. COI and COB sequences of a diphyllobothridean tapeworm *Diphyllobothrium latum* (GenBank accession no. AB269325) were used as outgroup.

Population structure and genetics of European and N. African lineages

Genealogy of obtained mtDNA haplotypes was reconstructed using TCS 1.21 (Clement et al. 2000). Statistics of genetic diversity (haplotype diversity—Hd; nucleotide diversity—Pi) and neutrality tests (Fu and Li's D and Tajima's D) were performed in DNASP 5.1 (Librado and Rozas 2009). Dataset containing combined COI and COB was also used to assess the level of population structure among studied samples performing Analysis of Molecular Variance (AMOVA) in Arlequin 3.5 (Excoffier and Lischer 2010). The calculations based on the F_{ST} coefficient (Weir and Cockerham 1984) were run using 10,000 permutations. Three levels of structure were evaluated independently for two Euro-Mediterranean clades A and B: (1) variability among European and North African populations, (2) variability among populations (localities) inside the two landmasses, and (3) variability among specimens inside each population.

Directionality of gene flow

Microsatellite data obtained by Štefka et al. (2007, 2009) were employed to test the hypothesis of Europe to Tunisia directionality of migration (i.e. invasion) of the clade A *L. intestinalis* populations. The dataset contains allelic data for 15 microsatellite loci in 189 specimens of European *Ligula* and 53 specimens of Tunisian *Ligula*. To detect directional gene flow, we

analysed the data for several contradicting structured population models using a Bayes factor approach (Jeffreys 1961; Kass and Raftery 1995) built into the program MIGRATE 3.2 (Beerli 2006; Beerli and Palczewski 2010). The Bayes factor analyses allow ordering of alternative, not necessarily nested population models. Bayes factors also take into account the available amount of data and the number of estimated parameters. In contrast, traditionally used likelihood ratio tests require nested models and do not correct for over-parameterization (Burnham and Anderson 2002). MIGRATE 3.2 implements thermodynamic integration to allow accurate model selection and model ordering. Model probabilities were calculated treating the marginal likelihoods like model weights (Kass and Raftery 1995), similar to the procedure described by Burnham and Anderson (2002) for Akaike's information criterion weights.

The microsatellite analysis of Štefka et al. (2009) using STRUCTURE software (Falush et al. 2007) did not indicate any structuring inside European populations of clade A *Ligula* despite their wide geographical range. To eliminate any effect that cryptic diversification among these populations could have on the Migrate analysis, the European samples were partitioned into 3 different sets and analysed independently against the Tunisian population with 53 samples. The first set contained a random sample of 75 plerocercoids from all European populations totalling 189 plerocercoids obtained by Štefka et al. (2009). The second set contained 84 French samples, which are geographically the closest sampled localities to Tunisia. For a comparison to the French samples, the third analysed set contained 58 plerocercoids from Czech localities, which are geographically more distant. Microsatellite datasets used in the analyses have been deposited in the Dryad data repository (10.5061/dryad.54fk2).

Migrate analyses were run using four population genetic models: (1) Four parameter model allowing for two different sizes for the northern and southern population and two independent migration parameters; (2) Three parameter model identical to (1) except that there is no migration from North to South; (3) Three parameter model identical to (1) except that there is no migration from South to North and (4) One parameter model that assumes that the northern and southern samples belong to the same panmictic population. Each set of models was run for the data pairs: France and Tunisia, Czech Republic and Tunisia, Europe and Tunisia. The models were then compared using their marginal likelihoods (Bayes factors: Kass and Raftery 1995; Beerli and Palczewski 2010).

Before running all models, we established run time parameters of MIGRATE so that replicated runs return the same marginal likelihoods plus minus 0.5 log likelihoods units. Each MIGRATE run used Bayesian inference with 4 parallel chains with temperatures 1.0, 1.5, 3.0, and 1,000,000. Likelihoods from these parallel chains were collected to approximate the marginal likelihood using thermodynamic integration (Beerli and Palczewski 2010). Run parameters were the following: 100,000 steps burn-in, sampling 10,000 parameters every 100th step for each of the 15 loci. Prior distributions were uniform and over the range of 0–100 for the mutation-scaled effective population size Theta (Θ) and 0–100 for the mutation-scaled effective migration rate (M). Brownian motion approximation to the stepwise microsatellite mutation model (introduced in Migrate version 1.5, 2002, Blum et al.2004) was used in the analysis.

Results

Epidemiological parameters of North African populations

According to our observations, the parasite *L. intestinalis* occurs in the two introduced species *R. rubilio* and *S. erythrophthalmus* populations in Sidi Salem and Nebhana dam reservoirs in Tunisia. Moreover, the parasite was also found to inhabit body cavities of the autochthonous fish *P. callensis* in Joumine and Remel sites. However, no specimen was found among the 120 dissected specimens of native *B. callensis* in sidi salem and Nebhana reservoirs (Table 3). Paradoxically, in Algeria, *Ligula* samples were found in native *Barbus* sp in Keddara, Hamiz and Taksebt reservoirs and in native *P. callensis* in Oubeira reservoir. In Algeria, *Ligula* was also recovered from introduced *Cyprinus carpio* and *Anguilla anguilla* (Anguillidae). Due to a long-term fixation in formalin, these specimens were unfortunately unsequenceable. This unsequenced material, probably belonging to the European clade A, is marked on the map in Fig. 1.

Sampling in Tunisia was extensive enough to allow epidemiological comparisons of infection between

Table 3 Prevalence and mean intensity of *L. intestinalis* in introduced and native host species in Tunisian freshwater

Fish species	Host + parasite status	Sampling Site	Number of inspected fish	Prevalence (%)	Mean intensity (MI)
R. rubilio	Introduced	Sidi Salem	256	14.8	2.9
		Nebhana	79	5.1	6.3
S. erythrophthalmus	Introduced	Sidi Salem	95	4.2	1.8
		Nebhana	72	1.4	4.0
B. callensis	Native	Sidi Salem	70	0.0	0.0
		Nebhana	50	0.0	0.0
P. callensis	Native	Sidi Salem	20	0.0	0.0
		Nebhana	15	0.0	0.0
		Joumine	105	4.8	2.0
		Remel	254	18.5	1.7

host species and localities. The values of prevalence and mean intensity of infection with *Ligula* plerocercoids are provided in Table 3. The data show that roach is more infected with *Ligula* than rudd in both studied sites where the introduced species co-occur. Prevalence (P) in Sidi Salem site is higher than that in Nebhana site, whereas the mean intensity (MI) is much lower in Sidi Salem than in Nebhana for both fish species.

Sequences and phylogenetic analysis

The concatenated matrix of COI and COB sequences was 801 bp long and contained data for 199 *Ligula* specimens. A total of 104 samples belonged to the Euro-Mediterranean populations from clades A and B, 43 new sequences were obtained in addition to earlier studies (Bouzid et al. 2008b). Eighty-four different haplotypes (23 new) were identified among the total of 199 analysed samples.

MP, ML and BI analyses of the concatenated matrix produced mutually congruent results with a robust nodal support for most clades (Fig. 2). Basic structure of the tree reflects geographical sampling of the specimens. Well separated lineages were found for samples from Europe, North Africa, Ethiopia and other geographical units. With the exclusion of MP, also the basal relationships among lineages from geographicaly separate regions are relatively well resolved compared to an earlier study analysing shorter stretch of mtDNA (Bouzid et al. 2008b). All sequenced *Ligula* specimens from the introduced roach and rudd in Sidi Salem and Nebhana were grouped in the same clade and clustered among the European specimens. *Ligula* specimens collected form autochtonous *P. callensis* were genetically distant from the introduced forms and clustered in the same clade as Algerian specimens from barbels (Fig. 2).

Population structure and genetics

The pattern of distribution of mtDNA haplotypes clearly shows common origin of European and Tunisian populations of the clade A *Ligula*. Tunisian haplotypes are dispersed across nearly the whole network of European samples. One haplotype (21) is even shared among Tunisian, French and Czech samples. An indication of emerging population structure is however visible with multiple Tunisian samples gathered in haplotypes 71, 73 and 74 on one side of the network. The clade B network shows higher degree of separation with none of the haplotypes shared between the two areas and with European haplotypes mostly centred in one part of the network. European clade B was sampled at much fewer localities than plerocercoids of the clade A, thus to a certain degree the genetic uniformity seen in European clade B samples may be due to unsampled populations.

Genetic diversity statistics show similar values of Hd for the clade A and B lineages (Table 4). The two lineages, however, differ markedly in their Pi values. Clade B showed an order of a magnitude higher value (0.0375) than clade A (0.0056). Similarly, the two clades differ in the results of the neutrality tests. Whilst clade A shows strongly negative values for both tests, clade B shows neutral results for Tajima's D but significantly positive value for Fu and Li's D. Several other differences emerge when populations

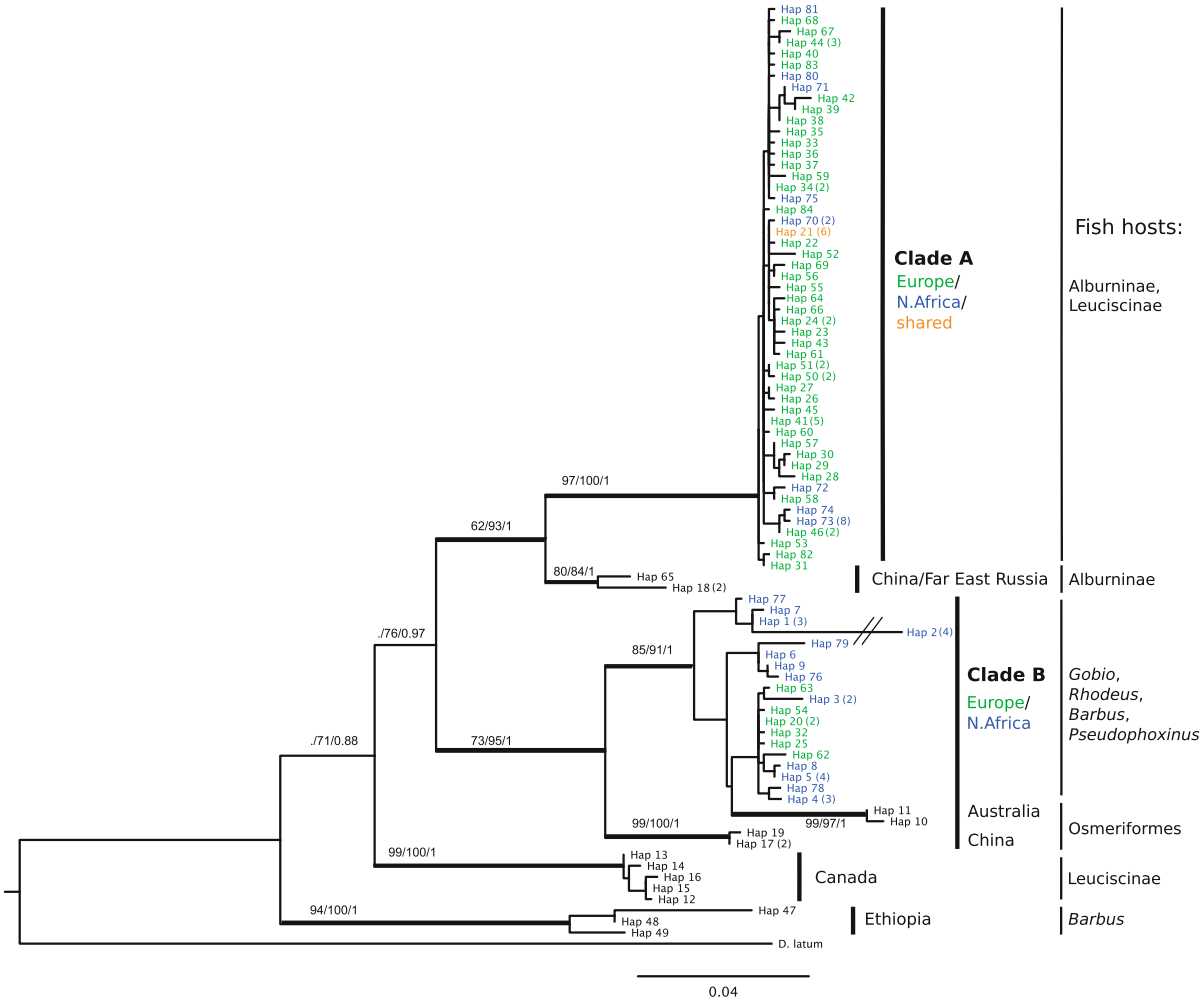


Fig. 2 Maximum likelihood tree based on concatenated matrix of sequences of cytochrome oxidase subunit I and cytochrome b. The numbers at the nodes indicate bootstrap support values higher than 60 % and posterior probabilities higher than 0.85 (MP/ML/BI). Haplotype numbers refer to the numbers listed in Table 1. *Numbers* in *parentheses* denote the number of samples grouped within a haplotype (if greater than one). Length of the haplotype 2 terminal branch was shortened

of the two lineages are analysed separately. Clade A populations from N. Africa show lower Hd and slightly lower Pi compared to the European population. N. African population shows neutral values of neutrality tests compared to highly negative results for Europe. Differences are seen also between European and N. African populations of clade B. European population show much lower value of Pi and slightly negative but significant values in both neutrality tests.

The results of AMOVA analysis correspond very well to the patterns seen in haplotype genealogies and in the diversity statistics. The volume of genetic variability captured at the highest level, between Europe and North Africa, shows moderate but significant values for both clades (Table 5). The value is lower for clade B, which may reflect a smaller number of samples analysed and shorter distances between some haplotypes. The highest volume of variability was however detected amongst samples within populations, which again corresponds with the patterns shown in Fig. 3.

Gene flow in the clade A

The MIGRATE analysis showed consistent results over all runs for the three arrangements of the data.

Table 4 Genetic diversity and neutrality tests in clade A and B populations from Europe and N. Africa

	N	H	Hd	Nd	Fu&Li's D	Tajima's D
CladeA	72	49	0.977	0.0056	−3.572**	−2.111**
CladeB	24	17	0.960	0.0375	1.393**	−0.020
CladeA-EU	56	42	0.984	0.0051	−4.044**	−2.289**
CladeA-N.Af.	16	8	0.758	0.0045	0.218	0.287
CladeB-EU	7	6	0.952	0.0036	−1.704*	−1.610*
CladeB-N.Af.	17	11	0.926	0.0475	1.544**	0.536

N number of samples, *H* number of haplotypes, *Hd* haplotype diversity, *Pi* nucleotide diversity. Levels of significance: ** ($P < 0.01$), * $P = <0.02–0.01>$

The direction of gene flow from Europe to Tunisia received the highest support (Table 6). Analyses of European subsets, France and Czech Republic, resulted in a similar model order. The best models were also those that allow for gene flow from North to South.

Discussion

Phylogeography of *Ligula* populations

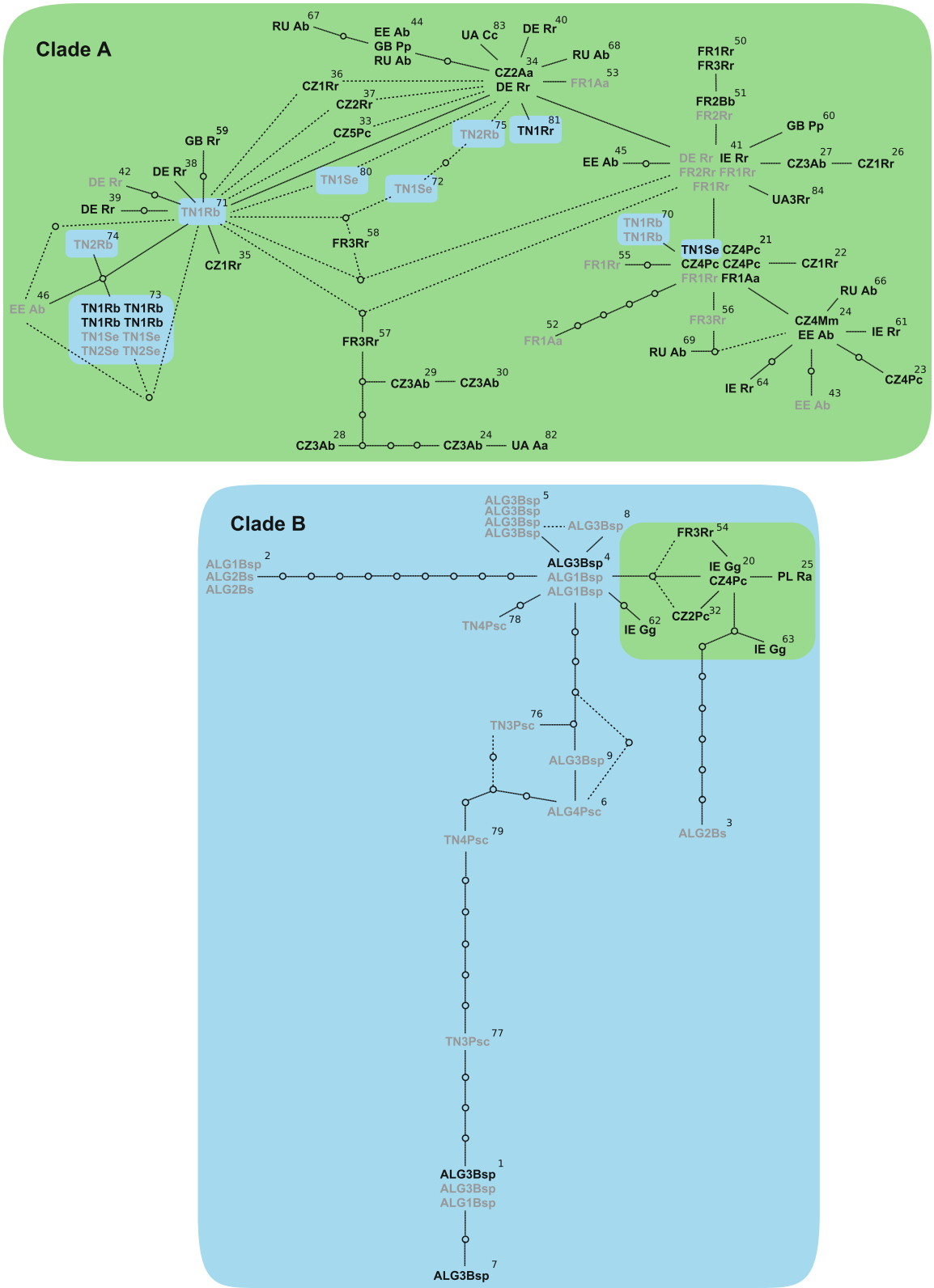
Species introductions are often followed by a rapid spread of introduced organisms throughout invaded areas (e.g. Fujisaki et al. 2010). Revealing invasion routes and the level of gene flow between introduced and parental populations may be obscured by the speed of dissemination of introduced populations and by insufficient knowledge of the genetic diversity of populations in the original distribution range (Khamis et al. 2009; Králová-Hromadová et al. 2011). The data and analyses presented in this study, successfully address these problems in the tapeworm species *L. intestinalis*. An earlier study by Bouzid et al. (2008b) showed that *L. intestinalis* is a globally distributed parasite forming multiple isolated lineages. Most of the lineages displayed localized allopatric distribution, but deeper evolutionary relationships between several of these lineages remained unresolved. Samples of two lineages, one from Mexico (Bouzid et al. 2008b) and one from China (Li et al. 2000; Li and Liao 2003), for which only COI data were available, have been removed from the current dataset compared to the earlier study. Nevertheless, because sequences of two mtDNA genes were combined in this study, the obtained phylogeny resolved the relationships amongst basal groups. Our results confirmed the basal position of the Ethiopian and Canadian lineages and placed the Euro-Mediterranean populations of *Ligula* (clades A and B) into a global context.

Two lineages with sympatric Euro-Mediterranean distribution are genetically highly differentiated and show a closer affinity to the samples outside their distribution range than to each other. Whereas the genetically uniform clade A is more closely related to samples morphologically identified as *L. interrupta* from China and Far East Russia, clade B comprises also samples from Australia and China (Fig. 2). This shows that the two clades are probably separated for evolutionary long time and are well adapted to their respective spectra of fish hosts. The situation inside these lineages is however different. While both lineages show strong genetic links between European and North African territories; this link is considerably stronger between the populations of the clade A. Despite the moderate level of population structure between the two continents revealed for both clades by AMOVA, this structure is probably only emerging in the clade A as indicated by the near even distribution of Tunisian haplotypes in Fig. 3. Surprisingly, the number of haplotypes in Tunisian populations is relatively high, showing that the pioneering population was considerably large. In accordance with this

Table 5 AMOVA results of population genetic structure in Euro-Mediterranean clades of *L. intestinalis* using mtDNA data

Level of hierarchy	Clade A				Clade B			
	F_{ST}	*df*	Variance components	%	F_{ST}	*df*	Variance components	%
1) Among continents	0.255	1	0.68755	25.48**	0.156	1	0.59438	12.97*
2) Among populations within continents	0.299	21	0.11835	4.39	0.266	8	0.62373	13.61
3) Within populations	0.059	51	1.89281	70.14**	0.130	19	3.36491	73.42

% Percentage of variation, Levels of significance: ** ($P < 0.001$), * $P = 0.006$


Clade A
RU Ab 67
EE Ab 44
GB Pp
RU Ab
UA Cc 83
DE Rr 40
RU Ab 68
CZ2Aa 34
FR1Aa 53
DE Rr
CZ1Rr 36
CZ2Rr 37
CZ5Pc 33
TN2Rb 75
TN1Rr 81
GB Rr 59
DE Rr 38
DE Rr 42
DE Rr 39
TN1Rb 71
TN1Se 80
TN1Se 72
TN2Rb 74
CZ1Rr 35
FR3Rr 58
EE Ab 46
TN1Rb TN1Rb
TN1Rb TN1Rb
TN1Se TN1Se
TN2Se TN2Se 73
FR3Rr 57
CZ3Ab 29
CZ3Ab 30
CZ3Ab 28
CZ3Ab 24
UA Aa 82
FR1Rr 50
FR3Rr
FR2Bb 51
FR2Rr
GB Pp 60
EE Ab 45
DE Rr IE Rr 41
FR2Rr FR1Rr
FR1Rr
CZ3Ab 27
CZ1Rr 26
UA3Rr 84
TN1Rb 70
TN1Rb
TN1Se CZ4Pc 21
FR1Rr 55
CZ4Pc CZ4Pc
FR1Rr FR1Aa
CZ1Rr 22
RU Ab 66
FR1Aa 52
FR3Rr 56
CZ4Mm 24
EE Ab
IE Rr 61
RU Ab 69
IE Rr 64
EE Ab 43
CZ4Pc 23
Clade B
ALG3Bsp 5
ALG3Bsp
ALG3Bsp
ALG3Bsp
ALG3Bsp 8
FR3Rr 54
ALG1Bsp 2
ALG2Bs
ALG2Bs
ALG3Bsp 4
ALG1Bsp
ALG1Bsp
IE Gg 20
CZ4Pc
PL Ra 25
TN4Psc 78
IE Gg 62
CZ2Pc 32
IE Gg 63
TN3Psc 76
ALG3Bsp 9
ALG4Psc 6
TN4Psc 79
ALG2Bs 3
TN3Psc 77
ALG3Bsp 1
ALG3Bsp
ALG1Bsp
ALG3Bsp 7

◀ **Fig. 3** Haplotype networks of analysed populations. *Hollow circles* represent missing haplotypes along the mutational pathway. *Dashed lines* mark alternate connections, each only 1 bp long. *Green background* highlights samples collected in Europe, *blue* samples from North Africa. Sequences obtained from GenBank are in black print, new sequences are in *grey*. Abbreviated names of analysed *Ligula* specimens and their respective haplotype numbers are the same as listed in Table 1. (Color figure online)

Table 6 Population model comparison in clade A: log marginal likelihoods for four models

Population pairs	1	2	3	4
France–Tunisia	−49,635	**−24,979**	−26,609	−25,970
Czech Rep.–Tunisia	−38,258	**−18,734**	−19,572	−19,579
Europe–Tunisia	−104,772	**−55,503**	−68,604	−57,173
Ranking	4,4,4	**1,1,1**	3,2,3	2,3,2
Model probability	0.0	**1.0**	0.0	0.0

The white circle marks the first population (European), black the second (Tunisian). Highest values are in bold

fact the Bottleneck analysis of Štefka et al. (2009) did not find significant genetic loss in these populations analysing multilocus data for all available specimens. Conversely, intercontinental population structure in clade B is probably of older origin with none of the haplotypes shared between Europe and North Africa.

The pattern of the two genetically differentiated clades with differing level of population subdivision between the two continents is also supported by the results of the summary statistics in Table 5. Whereas Bouzid et al. (2008b) were only able to compare the overall differences between the clade A and B lineages, extended sampling allowed us to contrast also intraclade differences between European and N. African populations of the two clades. Decreased value of Hd in clade A populations from Tunisia compared to Europe may be related to their recent introduction. Conversely, the N. African population of Clade B shows higher values of Pi compared to the samples from Europe. This may be either due to longer presence of the clade B in Africa or due to historical population bottlenecks in the European population reducing its genetic diversity, e.g. during quaternary glaciations. This view of different processes ongoing in European and N. African populations is also corroborated by the neutrality test results. When analysed together the clade B populations show weak sign of population size decrease or stagnation (positive value of Fu and Li's D test, near zero value in Tajima's D). When analysed separately, the N. African population retains the picture of a stagnating population (positive and close to zero values), whereas European population shows significantly negative values, which is usually interpreted as a sign of historical growth (Hartl and Clark 1997).

Multilocus analysis of gene flow

Despite a very short time since the suggested introduction of the clade A to the North African territory, a north to south direction of the gene flow across Mediterranean Sea was clearly confirmed by the analysis of multilocus genetic data. Based on the absence of the introduced *Ligula* in all 514 analyzed native fish from the Tunisian lakes, we are confident that no clade A specimens were present in North Africa prior to the introduction of European fish hosts in the late 1960s. The generation time in *Ligula* is typically between 20 and 36 months (Dubinina 1980), hence only up to 25 generations passed by since the introduction of the host populations to Tunisia 40–50 years ago. MIGRATE is based on the coalescence framework and estimates long-term parameters, but runs with simulated datasets (Beerli 2009) show that the most recent past contributes more heavily to the parameter estimates than the distant past. The estimated migration rates may be inflated by the recent divergence, but our results show that the differentiation of the Tunisian and the European population has progressed enough to estimate the direction of ancestral gene flow with confidence.

Host specificity and ecology of native and introduced parasite populations

Previous studies suggested mixed effect of geography and host specificity resulting in different host preferences of the two clades. Whereas clade A was found in a phylogenetically derived group of cyprinid fish (subfamilies Alburninae and Leuciscinae), clade B was found in basal groups of cyprinids like Gobioninae and Cyprininae (Bouzid et al. 2008b; Štefka et al.

2009). Thus, the host specificity of the two clades seemed to correlate with taxonomic affiliation of their host. Epidemiological data obtained here on an extended sampling in the North African region confirm such pattern only to a limited extent. The native African parasite fauna does not infect introduced hosts and, in the same way, the introduced populations are only restricted to the introduced hosts from the Leuciscinae subfamily. However, the clade B was frequently found both in basal cyprinids and in native North African Leuciscinae (*P. callensis*). This finding demonstrates that locally adapted populations or lineages of *Ligula* arise independently of the taxonomic origin of the hosts. Thus, the distribution of *Ligula* lineages in different host species is not driven by cospeciation as is common in many other groups of parasites or mutualists like ectoparasitic lice (e.g., Clayton et al. 2003; Light and Hafner 2008) and endosymbionts (Thao et al. 2000), respectively.

Speciation and diversification through host specificity is widely accepted as an important mode of speciation in parasitic organisms (Poulin 2007). The evolutionary patterns shown here demonstrate that for *L. intestinalis*, the geographic distribution is the key factor in the development of genetically isolated lineages and host specificity arises secondarily through adaptation to locally available hosts. Immunological interactions between the host and parasite were suggested as the factor defining the width of host specificity in *Ligula* (Arme 1997; Olson et al. 2002; Bouzid et al. 2008b). These adaptations however provide a very strong barrier against host switching. With exclusion of a single exception, both lineages retain their host specificity even in localities with sympatric occurrence of both types of cyprinid hosts. The exception was found by Štefka et al. (2009), who identified one of the specimens retrieved from roach using microsatellite genotyping and mtDNA sequencing as a member of the clade B. Similarly, the same specimen (haplotype 54, FR3Rr) clusters with other European clade B members in our analysis (Fig. 3).

In the analysis of host preferences of introduced *Ligula* populations, we found that roach is infected more frequently than rudd (Table 3). Surprisingly, rudd represents the predominant species (48 %) within the introduced fish community, whereas roach abundance is only 6 % (Djemali et al. 2003). Such discrepancy between parasite infection rates and host population densities is in contrast to the expectations predicting more common species or genotypes to be infected more frequently, a phenomenon sometimes referred to as coevolutionary alternation (Thompson 1994; Nuismer and Thompson 2006). We cannot exclude that current situation is only a temporary stage in the long-term cycle of population dynamics between *Ligula* and its hosts. For example, at the beginning of the fish introduction campaign in the 1960s, roach abundance in Sidi Salem reservoir was much higher than that of rudd (Kraïem 1983; Djemali 2005). Unfortunately, no historical data on *Ligula* prevalence in roach and rudd in Tunisia are available and we cannot conclude if there was a historical correlation between parasite prevalence and host abundance. However, such dynamics was described in a *Ligula* population infecting roach and rudd in Great Britain, where infection rates in a small lake population fluctuated between 0 and 78 % in approximately 10-year cycles (Kennedy et al. 2001). These fluctuations were assigned to the mixed effect of the mortality of infected fish, competition between the two host species and availability of definitive hosts. Additionally, Loot et al. (2006) showed that the similarities of the temporal dynamics of host life cycle act to favour or disadvantage the success of local host selection by parasites with a complex life cycle. The dynamics of roach in Tunisia could favour the encounter rate between roach populations and copepods as successive intermediate hosts, but other biological features such as differences in feeding preferences between roach and rudd may play an important role (for a discussion see Bahri-Sfar et al. 2010).

Concerns for local fish fauna

It is possible that the parasites of the clade A will eventually break the current host barrier between the introduced and native fish in North Africa and will become infective for *P. callensis*, which is phylogenetically and ecologically close species to the introduced hosts (Perea et al. 2010). Similarly, we cannot exclude that the clade B occurring in *P. callensis* will become infective for introduced hosts, although the situation appears more complex. Clade B is natively distributed both in North Africa and in Europe, and apart from the exception described above (one specimen found in roach in France); it retains specificity to the basal groups of cyprinids within its European

range despite sympatric occurrence of many other cyprinid hosts. Furthermore, the clade B comprises several haplotypic lineages, and based on microsatellite analysis of Štefka et al. (2009), the North African and European samples represent two related, but distinguishable clusters. Hence it is conceivable that each of these clusters possesses specific adaptations preventing successful host switches at least within a short period of time.

Despite restricted distribution of the clade A in introduced fish, there is concern that introduced populations of the parasite can spread to other Tunisian freshwater areas where potential *Ligula* host populations were introduced (reservoirs Ben Mtir, Bir Mechergua, etc.) by transfer of infected fish stock and/or by birds. In Algeria, several cases of parasite infection with *Chilodonella cyprini* (Ciliophora), *Gyrodactylus sp.*, *Dactylogyrus sp.*, *D. anchoratus* (Monogena) *Bothriocephalus acheilognathi* and *L. intestinalis* were reported following the introduction of cyprinids such as *Cyprinus carpio*, *Aristichthys nobilis*, *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* (Meddour 1988; Meddour et al. 2005). Besides, infection with *Ligula* may lead to serious changes in the trophic chain. Carnivorous species like the pikeperch *Sander lucioperca* that feed on the infected fish could experience significant changes in their dynamics. Djemali et al. (2003) showed that the population dynamics of the introduced rudd and roach strongly influence the state of the pikeperch stock. In addition, the reversal of the situation in roach and rudd abundance is likely to be attributable to a high perturbation and mortality of the roach stock infected by *Ligula* (Ben Hassine, personal communication). This is of great concern given that the pikeperch has an important national economic values but also international in that it is exported to European markets where the need in this species is increasing.

To conclude, using an example of a freshwater fish parasite we demonstrate that a combination of multilocus markers with advanced analytic tools helps to reconstruct invasion pathways even in organisms where the introduction to novel environment occurred only very recently. This approach also allows discrimination between cryptic lineages of the species in question, where native and introduced populations may otherwise be easily confused. This is important in order to better understand the course and impact of biological invasion. We combined these genetic data with epidemiological survey in the Tunisian area of distribution, which confirmed the validity of host-based division between the introduced and native populations of the parasite. Although this introduced parasite lineage does not represent immediate threat to the native fish fauna of the North African region, the information on the genetic links and distribution of the introduced parasite represents a solid baseline for future development of prevention and control of introduced species, known to harbour harmful parasites or pathogens.

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References

Arme C (1997) Ligulosis in two cyprinid hosts: *Rutilus rutilus* and *Gobio gobio*. Helmithologia 34:191–196

Ascunce MS, Yang C–C, Oakey J, Calcaterra L, Wu W-J, Shih C-J, Goudet J, Ross KG, Shoemaker DW (2011) Global invasion history of the fire ant *Solenopsis invicta*. Science 331:1066–1068

Bahri-Sfar L, Haddaoui N, Bouzid W, Essetti I, Qninba A, Ben Hassine OK (2010) Compared parasitic infection of *Ligula intestinalis* (Cestoda: Diphyllobothridae) in Cyprinidae species: *Rutilus rubilio* and *Scardinius erythrophthalmus* in two dam reserves in Tunisia. Parasite 17:241–250

Beerli P (2006) Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. Bioinformatics 22:341–345. doi:10.1093/bioinformatics/bti803

Beerli P (2009) How to use migrate or why are markov chain monte carlo programs difficult to use? In: Bertorelle G, Bruford MW, Hauffe HC et al (eds) Population genetics for animal conservation. Cambridge University Press, Cambridge, pp 42–79

Beerli P, Palczewski M (2010) Unified framework to evaluate panmixia and migration direction among multiple sampling locations. Genetics 185:313–326. doi:10.1534/genetics.109.112532

Blum MGB, Damerval C, Manel S, Francois O (2004) Brownian models and coalescent structures. Theor Popul Biol 65: 249–261

Bouzid W, Lek S, Mace M, Ben Hassine OK, Etienne R, Legal L, Loot G (2008a) Genetic diversity of *Ligula intestinalis* (Cestoda: Diphyllobothriidea) based on analysis of inter-simple sequence repeat markers. J Zool Syst Evol Res 46:289–296

Bouzid W, Štefka J, Hypša V, Lek S, Scholz T, Legal L, Ben Hassine OK, Loot G (2008b) Geography and host specificity: two forces behind the genetic structure of the freshwater fish parasite *Ligula intestinalis* (Cestoda: Diphyllobothriidae). Int J Parasitol 38:1465–1479

Brown JE, Stepien CA (2010) Population genetic history of the dreissenid mussel invasions: expansion patterns across North America. Biol Invasions 12:3687–3710

Brown SP, Loot G, Grenfell BT, Guégan JF (2001) Host manipulation by *Ligula intestinalis*: accident or adaptation? Parasitology 123:519–529

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach, 2nd edn. Springer, New York

Carter V, Pierce R, Dufour S, Arme C, Hoole D (2005) The tapeworm *Ligula intestinalis* (Cestoda: Pseudophyllidea) inhibits LH expression and puberty in its teleost host, *Rutilus rutilus*. Reproduction 130:939–945

Clavero M, García-Berthou E (2005) Invasive species are a leading cause of animal extinctions. Trends Ecol Evol 20:110. doi:10.1016/j.tree.2005.01.003

Clayton DH, Al-Tamimi S, Johnson KP (2003) The ecological basis of coevolutionary history In: Page RDM (ed) Tangled trees: phylogeny, cospeciation and coevolution. University of Chicago Press, Chicago, pp 310–341

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Mol Ecol 9:1657–1659

Dejen E, Vijverberg J, Sibbing FA (2006) Spatial and temporal variation of cestode infection and its effects on two small barbs (*Barbus humilis* and *B. tanapelagius*) in Lake Tana, Ethiopia. Hydrobiologia 556:109–117

Djemali I (2005) Évaluation de la biomasse piscicole dans les plans d'eau douce tunisiens: approches analytique et acoustique. Institut National Agronomique de Tunisie

Djemali I, Kraïem MM, Cadic N, Proteau JP, El Abed A, Jarboui O (2003) Evaluation de la biomasse piscicole en eau douce par écho prospection: application à la retenue de Sidi Salem. Bull Inst Natl Sci Technol Mer 30:23–32

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449. doi:10.1111/j.1365-294X.2007.03538.x

Dubinina MN (1980) Tapeworms (Cestoda, Ligulidae) of the fauna of the USSR. Amerind Publishing Co, New Delhi

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567. doi:10.1111/j.1755-0998.2010.02847.x

Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol Ecol Notes 7:574–578

Fujisaki I, Pearlstine EV, Mazzotti FJ (2010) The rapid spread of invasive Eurasian collared doves *Streptopelia decaocto* in the continental USA follows human-altered habitats. Ibis 152:622–632

Goka K, Yokoyama J, Une Y, Kuroki T, Suzuki K, Nakahara M, Kobayashi A, Mizutani T, Inaba S, Hyatt AD (2009) Amphibian chytridiomycosis in Japan: distribution, haplotypes and possible route of entry into Japan. Mol Ecol 18:4757–4774

Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

Hartl DL, Clark AG (1997) Principles of population genetics, 3rd edn. Sinauer Associates, Sunderland, MA

Hoole D, Carter V, Dufour S (2010) *Ligula intestinalis* (Cestoda: Pseudophyllidea): an ideal fish-metazoan parasite model? Parasitology 137:425–438

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17:754–755

Jeffreys H (1961) The theory of probability. Oxford University Press, New York. (3rd edition 1998)

Kass RE, Raftery AE (1995) Bayes Factors. J Am Stat Assoc 90:773–779

Kennedy CR, Shears PC, Shears JA (2001) Long-term dynamics of *Ligula intestinalis* and roach *Rutilus rutilus*: a study of three epizootic cycles over thirty-one years. Parasitology 123:257–269

Khalil L, Polling L (1971) Checklist of the helminth parasites of african freshwater fishes. In: Farnham R (ed). Commonwealth Agricultural Bureaux publishing, Slough, pp 80

Khamis FM, Karam N, Ekesi S, De Meyer M, Bonomi A, Gomulski LM, Scolari F, Gabrieli P, Siciliano P, Masiga D, Kenya EU, Gasperi G, Malacrida AR, Guglielmino CR (2009) Uncovering the tracks of a recent and rapid invasion: the case of the fruit fly pest *Bactrocera invadens* (Diptera: Tephritidae) in Africa. Mol Ecol 18:4798–4810

Kraïem M (1983) Les poissons d'eau douce de Tunisie: inventaire commenté et répartition géographique. Bull Inst Natl Sci Tech Océanogr Pêche Salammbô 10:107–124

Kraïem M (1991) Étude Eco-biologique de principales espèces de poissons des retenues des barrages de Sidi Salem, Bir M'Cherga, Mellègue, Bouhertma, Nebhana et Sidi Saad. Rapports Techniques Projet Tuniso-Allemend de pêche continentale 11: 48

Králová-Hromadová I, Bazsalovicsová E, Štefka J, Špakulová M, Vávrová S, Szemes T, Tkach V, Trudgett A, Pybus M (2011) Multiple origins of European populations of the giant liver fluke *Fascioloides magna* (Trematoda: Fasciolidae), a liver parasite of ruminants. Int J Parasitol 41: 373–383

Li J, Liao X (2003) The taxonomic status of *Digramma* (Pseudophyllidae: Ligulidae) inferred from DNA sequences. J Parasitol 89:792–799

Li J, Liao X, Yang H (2000) Molecular characterization of a parasitic tapeworm (*Ligula*) based on DNA sequences from formalin-fixed specimens. Biochem Genet 38:309–322

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

Light JE, Hafner MS (2008) Codivergence in heteromyid rodents (Rodentia: Heteromyidae) and their sucking lice of

the genus *Fahrenholzia* (Phthiraptera: Anoplura). Syst Biol 57:449–465

Loot G, Lek S, Brown SP, Guégan J-F (2001a) Phenotypic modification of roach (*Rutilus rutilus* L.) infected with *Ligula intestinalis* L. (Cestoda: Pseudophyllidea). J Parasitol 87:1002–1010

Loot G, Lek S, Dejean D, Guégan J-F (2001b) Parasite-induced mortality in three host populations of the roach *Rutilus rutilus* (L.) by the tapeworm *Ligula intestinalis* (L.). Ann Limnol Int J Limnol 37:151–159

Loot G, Aulagnier S, Lek S, Thomas F, Guégan J-F (2002) Experimental demonstration of a behavioural modification in a cyprinid fish, *Rutilus rutilus* (L.), induced by a parasite, *Ligula intestinalis* (L.). Can J Zool 80:738–743

Loot G, Park Y-S, Lek S, Brosse S (2006) Encounter rate between local populations shapes host selection in complex parasite life cycle. Biol J Lin Soc 89:99–106

Losse G, Nau W, Winter M (1991) Le développement de la pêche en eau douce dans le nord de la Tunisie: Projet de la coopération technique Tuniso-Allemande. Utilisation des barrages pour la pisciculture, GTZ Gmbh

Manilla G, Albertini D, Falasca MP (1984) *Ligula intestinalis* (L., 1758) Gmelin, 1790 (Cestoda: Ligulidae) in *Rutilus rubilio* (Pisces: Cyprinidae) of Campotosto Lake. Riv Parasitol 45:263–279

Margolis L, Esch GW, Holmes JC, Kuris AM, Schad GA (1982) The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). J Parasitol 68:131–133

Meddour A (1988) Parasites of freshwater fishes from Lake Oubeira. Dissertation, Department of Zoology, The University of Liverpool, UK, Algeria

Meddour A, Rouabhi A, Meddour-Bouderda K, Loucif N, Remili A, Khataly Y (2005) Expérimentations sur la reproduction artificielle de *Sander lucioperca*, *Hypophthalmichthys molitrix* et *Aristichthys nobilis* en Algérie. Sci Technol 23:63–71

Mergeay J, Verschuren D, De Meester L (2006) Invasion of an asexual American water flea clone throughout Africa and rapid displacement of a native sibling species. Proc R Soc Lond Biol 273:2839–2844

Nei M, Kumar S (2000) Molecular evolution and phylogenetics. Oxford University Press, New York

Nuismer SL, Thompson JN (2006) Coevolutionary alternation in antagonistic interactions. Evolution 60:2207–2217

Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Center Uppsala University

Nylander JAA, Wilgenbusch JC, Warren DL, Swofford DL (2008) AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24:581–583

Olson PD, Littlewood DTJ, Griffiths D, Kennedy CR, Arme C (2002) Evidence for the co-existence of separate strains or species of *Ligula* in Lough Neagh, Northern Ireland. J Helminthol 76:171–174

Perea S, Böhme M, Zupancic P, Freyhof J, Sanda R, Özulug M, Abdoli A, Doadrio I (2010) Phylogenetic relationships and biogeographical patterns in Circum-Mediterranean subfamily Leuciscinae (Teleostei, Cyprinidae) inferred from both mitochondrial and nuclear data. BMC Evol Biol 10:265

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol Econ 52:273–288

Posada D (2004) Collapse (Version 1.2). A tool for collapsing sequences to haplotypes. http://darwin.uvigo.es/

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817–818. doi:10.1093/bioinformatics/14.9.817

Poulin R (2007) Evolutionary ecology of parasites, 2nd edn. Princeton University Press, Princeton

Prenter J, Macneil C, Dick JTA, Dunn AM (2004) Roles of parasites in animal invasions. Trends Ecol Evol 19:385–390

Rambaut A, Drummond A (2005) Tracer. Institute of Evolutionary Biology, University of Edinburgh. http://tree.bio.ed.ac.uk/software/tracer/

Rushton SP, Lurz PWW, Gurnell J, Nettleton P, Bruemmer C, Shirley MDF, Sainsbury AW (2006) Disease threats posed by alien species: the role of a poxvirus in the decline of the native red squirrel in Britain. Epidemiol Infect 134:521–533

Sasal P, Taraschewski H, Grondin H, Valade P, Wielgoss S, Moravec F (2008) Parasite communities in eels of the Island of Reunion (Indian Ocean): a lesson in parasite introduction. Parasitol Res 102:1343–1350

Štefka J, Gilleard JS, Grillo V, Hypša V (2007) Isolation and characterization of microsatellite loci in the tapeworm *Ligula intestinalis* (Cestoda: Pseudophyllidea). Mol Ecol Notes 7:794–796

Štefka J, Hypša V, Scholz T (2009) Interplay of host specificity and biogeography in the population structure of a cosmopolitan endoparasite: microsatellite study of *Ligula intestinalis* (Cestoda). Mol Ecol 18:1187–1206

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Thao ML, Moran NA, Abbot P, Brennan EB, Burckhardt DH, Patrick A, Brennan EB, Burckhardt DH, Baumann P (2000) Cospeciation of psyllids and their primary prokaryotic endosymbionts. Appl Environ Microbiol 66: 2898–2905

Therriault TW, Orlova MI, Docker MF, Macisaac HJ, Heath DD (2005) Invasion genetics of a freshwater mussel (*Dreissena rostriformis bugensis*) in eastern Europe: high gene flow and multiple introductions. Heredity 95:16–23

Thompson JN (1994) The coevolutionary process. University of Chicago Press, Chicago

Van Riper C III, van Riper SG, Goff ML, Laird M (1986) The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecol Monogr 56:327–344

Wattier RA, Haine ER, Beguet J, Martin G, Bollache L, Muskó IB, Platvoet D, Rigaud T (2007) No genetic bottleneck or associated microparasite loss in invasive populations of a freshwater amphipod. Oikos 116:1941–1953

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370

Wielgoss S, Taraschewski H, Meyer A, Wirth T (2008) Population structure of the parasitic nematode *Anguillicola crassus*, an invader of declining North Atlantic eel stocks. Mol Ecol 17:3478–3495