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Parasite and genetic diversity of big-scale sand smelt (*Atherina boyeri* Risso, 1810) populations in their natural and expansion ranges in Ukraine

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Abstract

The big-scale sand smelt (*Atherina boyeri*) is an Atlanto-Mediterranean amphidromous fish species found within the Black Sea. Here, we assess differences in the parasite fauna of big-scale sand smelt populations from their natural range in the northwestern Black Sea and from their expansion range in the Lower and Middle River Dnipro. In addition, we undertook a microsatellite analysis to assess the genetic similarity of fish from the different locations. We found that the parasite community of fish in their natural range was wider than that from their expansion range. While the Gulf of Odesa was most distant from all other localities by parasite community composition and the Dnipro Reservoir was characterised by an absence of parasites (newest and most distant expansion locality), only fish from the Danube Delta showed a significant genetic difference. Our results suggest that the parasite community of big-scale sand smelt is primarily influenced by environmental factors, such as habitat type, water salinity and/or prey composition. Both microsatellite analysis and parasite community species composition (e.g. the presence of the marine *Telosentis exiguus* in the Kakhovka Reservoir and freshwater *Raphidascaris* sp. in the Gulf of Odesa) confirmed that populations in the River Dnipro reservoirs had, at some time, been connected with native marine populations, thus also confirming the species' amphidromous nature.

Keywords Population genetics · Parasite community · Fish · Neolimnetics · Microsatellites · Genetic diversity · River Dnipro

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Introduction

Ukraine is situated on the crossing of several transport corridors, making it a hub for the spread of alien species, with two important routes for aquatic invasive species, the so-called Southern and Central corridors, meeting here (Panov et al. 2009). These corridors have allowed both distant aliens, introduced from remote geographic regions, and neighbouring aliens, to increase their ranges within the same geographic region (Alexandrov et al. 2007). The terminology describing such invasions has received much attention in recent years (e.g. Copp et al. 2005); however, most of these studies have focused on the distribution of Ponto-Caspian gobiids (Gobiidae) in Northern and Southern Europe (see Kvach et al. 2021). It should be noted that there are many other Ponto-Caspian species increasing their ranges along these corridors. For example, in recent years, the riverine systems of Ukraine have been invaded by a group known as neolimnetics, i.e. species that originate in brackish waters

but are able to increase their range by invading upstream stretches of freshwater rivers (Kvach and Kutsokon 2017).

One such neolimnetic species is the big-scale sand smelt (*Atherina boyeri* Risso, 1810; Actinopterygii: Atherinidae; Kvach and Kutsokon 2017), an Atlanto-Mediterranean species that naturally inhabits the eastern Atlantic from the British Isles and the Netherlands in the north to Mauritania and Madeira in the south, including the Mediterranean and Black Seas (Quignard and Pras 1986). Freshwater populations are known from the Santo André lagoon on the Iberian Peninsula, Lake Trichonis in Greece and Lake Trasimeno in Italy (Kottelat and Freyhof 2007). In Italy, the species' range has expanded into many lakes, most likely through accidental introductions (Moretti et al. 1959; Bianco et al. 2013). In the Black Sea basin, the fish has been translocated into several natural lakes in Central Anatolia (Turkey), reservoirs in the Kizilirmak river basin and some parts of the Dardanelles Straight and Mediterranean drainage basins (Partal et al. 2019). In Ukraine, the species has penetrated as far as the River Dnipro (Dnieper) and has established populations in the Kakhovka, Dnipro and Kamianske reservoirs (Marenkov 2018; Zhukinsky et al. 2007).

Small founding populations, originating from a single introduction, have less variable genotype diversity than populations in their natural range, in part due to 'bottleneck effects' or gene drift (Bock et al. 2015). Conversely, multiple introductions significantly increase the success of invasive populations as each new introduction increases the genetic diversity of the introduced population (Roman and Darling 2007). In doing so, they reduce the negative effects of inbreeding caused by a lack of natural selection (Roman and Darling 2007), and, as a result, some alien populations can show higher genetic diversity than native populations of the same species (Kolbe et al. 2008).

In invaded ecosystems, non-native species that have been freed from their natural enemies, e.g. parasites, may be competitively superior to native species, leading to increased population growth due to a lack of spatial regulation (Keane and Crawley 2002). Likewise, native species will have to deal with a full range of pathogens, while alien species, as a rule, tend to have a lower number of natural enemies and a lower proportion of infected individuals (Torchin et al. 2003). The effects of this 'parasite release', or more controversially, of 'co-introduction of parasites with the host', will depend on different factors, including the size of the founding population (single or multiple introductions), its origin (from source regions with rich/poor parasite fauna) and/or its developmental stage (eggs, larvae or adults). In the case of co-introduction, the parasites ability to spread will also reflect the size of the founding population. Furthermore, any successful introduction of parasites with complex life cycles will require the presence of all components of its life cycle in the recipient ecosystem (MacLeod et al. 2010). In many

cases, alien species acquire local parasites and become new competent hosts (Tompkins et al. 2011). In doing so, these new hosts could amplify the transmission dynamics of native parasites, thereby increasing infection levels for native hosts (Kelly et al. 2009).

The aim of the present study was to investigate the parasite community of natural and neolimnetic populations of big-scale sand smelt in different Ukrainian habitats, also characterising the host genetic lineage.

## Material and methods

Fish sampling

The fish in this study were sampled from the species' natural range in the northwestern Black Sea and from within its expansion range in the Lower and Middle River Dnipro (see Fig. 1) from July to September 2021 (warm period). All specimens from each locality were caught on the same day and from the same geographical coordinates. Five sites were sampled in total, i.e.:

1) Solonyi Kut Bay in the Danube Delta; 45.472490N, 29.650889E (natural range)
2) Near Cape Malyi Fontan, Gulf of Odesa; 46.441210N, 30.772868E (natural range)
3) Village of Rybakivka, near Cape Adzhiyask, Dnipro Estuary forefront; 46.616132N, 31.376388E (natural range)
4) Village of Mylove, Kakhovka Reservoir, Lower Dnipro, 47.048524N, 33.651200E (expansion)
5) Village of Stari Kodaky, Dnipro Reservoir, Middle Dnipro, 48.370007N, 35.148804E (expansion)

The fish were caught using a 10-m seine with a 6-mm bar mesh size. All fish were immediately transported to the laboratories alive in aerated water (taken from the place of sampling), where they were placed into aerated aquaria to await examination.

Parasitological analysis

In total, 76 fish were examined for parasites (Table 1). All fish were dissected within 3 days of sampling to ensure maximum parasite recovery (see Kvach et al. 2016). Prior to dissection, each fish was measured for standard (SL, mm) and total length (TL, mm). The dissection of larger number of fish was not possible, because the big-scale sand smelt is very sensitive to transportation, quickly dying because of stress. Because the distance between localities is quite high, the transportation and holding of alive specimens was problematic. The additional sampling is impossible now, because
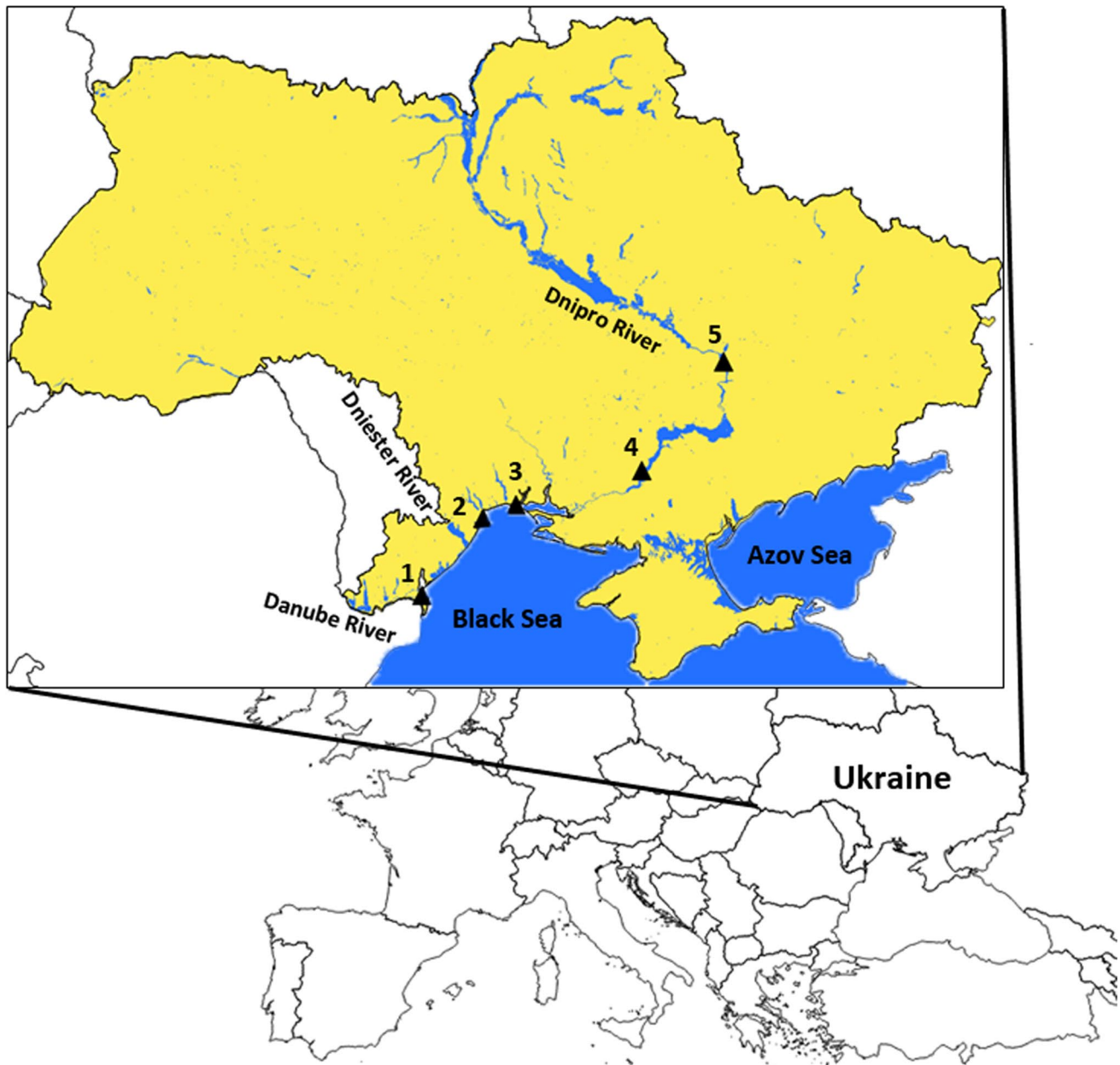
Fig. 1 Schematic map of the study area. Sampling localities are marked with triangles: 1 = Danube Delta; 2 = Gulf of Odesa; 3 = Cape Adzhiyask; 4 = Kakhovka Reservoir; 5 = Dnipro Reservoir

Table 1 Numbers of big-scale sand smelt (*Atherina boyeri*) examined in this study from native and expansion localities in Ukraine

Parameters		Natural range			Expansion range	
		Danube Delta	Gulf of Odesa	Cape Adzhiyask	Kakhovka Reservoir	Dnipro Reservoir
No.	Total	15	20	15	15	11
	DNA study	10	10	10	10	10
SL, mm	m ± sd	55.0 ± 21.9	57.4 ± 9.6	64.7 ± 6.9	62.4 ± 2.7	37.8 ± 8.7
	min–max	30–86	43–81	54–77	59–68	26–51
TL, mm	m ± sd	62.6 ± 25.3	66.6 ± 11.7	73.3 ± 7.0	70.6 ± 2.6	43.0 ± 10.1
	min–max	35–99	49–95	62–86	68–78	29–58

of Russian Invasion in Ukraine: several sampling localities are on occupied territory, the Black Sea and low Dnipro ecosystem is totally changed because of the Kakhovka disaster.

For each specimen, the fins, skin, gills, muscle tissue and internal organs were examined for presence of parasites. Unicellular parasites were studied live using light microscopy. Monogeneans were preserved in Glycerine-ammonium-picrate (GAP) and prepared as semi-permanent slides (Malmberg 1970), while digeneans, cestodes and nematodes were preserved in hot 4% formaldehyde solution and stained with iron acetic carmine, dehydrated in ethanol of increasing concentration and mounted in Canada balsam as permanent slides (Georgiev et al. 1986; Cribb and Bray 2010). Acanthocephalans were preserved in 70% ethanol, compressed between two glass slides and then mounted in glycerol as temporary slides for light microscopy. All parasites were identified to species or to genus if identification to species was not possible.

Identification keys were used to identify parasites to species level, or to the lowest possible taxa, based on specific morphological features. Microsporideans were identified alive based on morphology of xenomas and spores (Ovcharenko et al. 2017), while the morphological description provided by Kvach et al. (2019) was used to identify monogeneans and that of Moravec (2013) to identify nematodes. Other parasites were identified using the key provided in Gayevskaya et al. (1975). Scientific names and taxonomy were according to WoRMS (2023).

Indices of prevalence (P, %), intensity range (IR), mean intensity (MI) and mean abundance (A) were calculated for each parasite species and each locality (Bush et al. 1997), with the Czekanowski-Sørensen Index (CSI), presented as a percentage, used to compare the parasite fauna (CSI parameters > 50% considered significant; Sørensen 1948). The Shapiro-Wilk test was used to assess normality of fish size, and the t-test used to assess differences in fish from different localities. Finally, discriminant analysis was used to distinguish fish from different localities by parasite abundance. All statistical analyses were undertaken using StatSoft Statistica for Windows 10.

Molecular study

Material for the molecular study was obtained from 50 bigscale sand smelt sampled from the River Dnipro drainage, i.e. the Dnipro Reservoir (10 ind.) and the Kakhovka Reservoir (10 ind.), and from the Black Sea drainage, i.e. the Gulf of Odesa (10 ind.), Cape Adzhiyask near-shore (10 ind.) and the Danube Delta (10 ind.) (Table 1).

Genomic DNA was extracted from fin clips (preserved in 96% ethanol), based on the method of Sambrook and Russell (2001), using cetyl trimethylammonium bromide (CTAB). Each population was then analysed via PCR using three species-specific microsatellite markers (*AthB5*, *AthF1* and *AthD6*), as recommended by Milana et al. (2009). The PCR amplification products were then analysed by 7% polyacrylamide gel electrophoresis (Sambrook and Russell 2001). Silver nitrate (AgNO$_3$) staining was then used to visualise the amplification fragments in polyacrylamide gel, according to the Promega Technical Manual (1999).

Genetic diversity between fish populations from different locations was assessed using microsatellite markers and allele frequencies, number of effective and different alleles, Shannon's information index, observed and expected heterozygosity, fixation index and Nei genetic distance and identity, all calculated using GenAlEx v.6.5 (Nei 1978, 1987; Nei and Li 1979; Peakall and Smouse 2012). A dendrogram of the study populations was then constructed using the paleontological statistics software package for education and data analysis (PAST) (Hammer et al. 2001).

Results

The t-test showed that both SL and TL of fish from the Dnipro Reservoir differed significantly from that at all other localities ($p < 0.05$). While fish from Cape Adzhiyask had a significantly higher SL than those from the Danube Delta ($t = 2.48$, df $= 33$, $p < 0.05$), there was no significant difference in TL ($t = 1.99$, df $= 33$, $p < 0.05$). In all other cases, no significant differences were observed in fish size between localities (Table 1).

Parasite community

The total parasite fauna comprised 11 species, including one microsporidian, one ciliate, one monogenean, one cestode, four digeneans, one acanthocephalan and two nematode species (Table 2). Highest parasite richness was recorded from the Gulf of Odesa (eight species) and the Danube Delta (seven species), and lowest richness in the Kakhovka Reservoir (two species), with levels near cape Adzhiyask being intermediate (five species). No parasites were found on fish from the Dnipro Reservoir; consequently, these data are not included in Table 2. The highest difference in parasite fauna occurred between the Danube Delta, Gulf of Odesa and Cape Adzhiyask (Table 3), differences between Kakhovka Reservoir and the other study sites being < 50%.

Parasite abundance was non-normally distributed in all cases (Shapiro-Wilk test). While the parasite community from the Gulf of Odesa was significantly different compared to all other study localities ($p < 0.05$; Table 3), no significant differences were observed between communities from Cape Adzhiyask and the Danube Delta and between Cape Adzhiyask and Kakhovka Reservoir.

Table 2 Parasitation of big-scale sand smelt (*Atherina boyeri*) from native and expansion localities in Ukraine

Parasite species	Site	Index	Natural range			Expansion range
			Danube Delta	Gulf of Odesa	Cape Adzhiyask	Kakhovka Reservoir
Microsporidia						
Loma sp.	Gut wall	P, %	6.7	5.0	20.0	
		MI ± sd	1.0	10.0	4.7 ± 4.6	
		IR	1	10	2–10	
		A	0.1	0.5	0.9	
Ciliata						
Trichodina sp.	Fins	P, %	6.7	10.0	33.3	
		MI ± sd	1.0	1.0 ± 0.0	2.8 ± 4.0	
		IR	1	1	1–10	
		A	0.1	0.1	0.9	
Monogenea						
Gyrodactylus ginestrae	Fins	P, %		20.0		
		MI ± sd		1.3 ± 0.5		
		IR		1–2		
		A		0.3		
Cestoda						
Ligula sp.	Coelom	P, %				6.7
		MI ± sd				1.0
		IR				1
		A				0.1
Digenea						
Bucephalus marinum	Fins	P, %	6.7			
		MI ± sd	1.0			
		IR	1			
		A	0.1			
Bacciger bacciger	Gut	P, %		5.0		
		MI ± sd		3.0		
		IR		3		
		A		0.2		
Timoniella imbutiformis	Muscles	P, %	53.3	30.0	40.0	
		MI ± sd	3.9 ± 2.3	2.0 ± 0.9	2.0 ± 1.5	
		IR	1–7	1–3	1–5	
		A	2.1	0.6	0.8	
Cryptocotyle jujuna	Fins, skin	P, %		15.0	20.0	
		MI ± sd		3.0 ± 1.7	3.0 ± 1.7	
		IR		1–4	2–5	
		A		0.5	0.6	
Acanthocephala						
Telosentis exiguus	Gut	P, %	20.0	45.0	20.0	13.3
		MI ± sd	3.3 ± 2.5	2.9 ± 1.4	1.0 ± 0.0	2.0 ± 1.4
		IR	1–6	1–5	1	1–3
		A	0.7	1.3	0.2	0.3
Nematoda						
Eustrongylides sp.	Liver	P, %	6.7			
		MI ± sd	1.0			
		IR	1			
		A	0.1			

Table 2 (continued)

Parasite species	Site	Index	Natural range			Expansion range
			Danube Delta	Gulf of Odesa	Cape Adzhiyask	Kakhovka Reservoir
Raphidascaris sp.	Liver, mesentery	P, %	46.7	5.0		
		MI ± sd	3.9 ± 2.0	1.0		
		IR	1–7	1		
		A	1.8	0.1		
Species richness			7	8	5	2

Microsatellite analysis

Microsatellite analysis revealed a total of 77 different alleles, with high genetic variability between populations. For the *AthF1*, *AthB5* and *AthD6* loci, 26, 30 and 21 alleles were detected, respectively. The number of alleles per locus in each population ranged from 7 to 14, with an average of 10.4 alleles/locus (Table 4). The average number of effective alleles per locus was 7.2 in each population, while the allele size range ranged from 142 to 287 bp at the *AthF1* locus, 195 to 372 bp at the *AthB5* locus and 110 to 226 bp at the *AthD6* locus. Mean level of observed heterozygosity was similar between populations,

Table 3 Differences between big-scale sand smelt (*Atherina boyeri*) parasite communities from native and expansion localities in Ukraine

Site	Index	Natural range			Expansion range
		Danube Delta	Gulf of Odesa	Cape Adzhiyask	Kakhovka Reservoir
Danube Delta	CSI	100.00			
	MD	< 0.001			
	F				
	p*p*				
Gulf of Odesa	CSI	**66.67**	100.00		
	MD	**10.61**	< 0.001		
	F	**6.91**			
	p	< 0.001			
Cape Adzhiyask	CSI	**66.67**	**76.92**	100.00	
	MD	2.18	**8.32**	< 0.001	
	F	1.42	**4.74**		
	p	0.19	< 0.001		
Kakhovka Reservoir	CSI	22.22	20.00	28.57	100.00
	MD	**3.33**	**10.78**	2.64	< 0.001
	F	**2.17**	**6.14**	1.51	
	p	0.03	< 0.001	0.16	

Bold = significant differences ($p \leq 0.05$ or CSI > 50%)

CSI Czekanowski-Sørensen Index, *MD* Mahalanobis distance, *F* Fisher's criterion

Table 4 Genetic characteristics of big-scale sand smelt (*Atherina boyeri*) populations from native and expansion localities in Ukraine, revealed using microsatellite analysis

Genotype parameters	Danube Delta	Gulf of Odesa	Cape Adzhiyask	Kakhovka Reservoir	Dnipro Reservoir	Total
Different allele number	9.33 ± 0.88	10.3 ± 0.7	10.67 ± 1.76	10.67 ± 1.86	11.00 ± 1.00	10.40 ± 0.52
Effective allele number	6.23 ± 1.38	7.12 ± 1.25	8.73 ± 1.75	6.81 ± 2.00	7.34 ± 1.62	7.24 ± 0.65
Shannon's information index	1.99 ± 0.16	2.13 ± 0.13	2.23 ± 0.19	2.05 ± 0.30	2.15 ± 0.20	2.11 ± 0.08
Observed heterozygosity	0.93 ± 0.07	0.73 ± 0.03	0.70 ± 0.01	0.67 ± 0.033	0.80 ± 0.10	0.77 ± 0.03
Expected heterozygosity	0.82 ± 0.04	0.85 ± 0.03	0.88 ± 0.03	0.81 ± 0.08	0.84 ± 0.04	0.84 ± 0.02
Fixation index	0.15 ± 0.13	0.13 ± 0.07	0.20 ± 0.03	0.15 ± 0.11	0.05 ± 0.10	0.08 ± 0.05

ranging from 0.67 to 0.93. Overall, the observed heterozygosity was lower than expected for all loci in all populations, with the average value per locus being 0.77 and 0.84, respectively (Table 4). Shannon's information index and the effective number of alleles were highest for the Cape Adzhiyask population, being 2.23 and 8.73, respectively, indicating a higher genetic diversity in this population.

Allele frequencies for each microsatellite locus indicated that the alleles of 211 bp, 221 bp and 237 bp occurred with a maximum frequency of 0.2 in the *AthF1* locus, while the most common alleles were 112 bp and 218 bp at the *AthD6* and *AthB5* loci, with a frequency 0.55 and 0.45, respectively. While it was possible to observe alleles that distinguished each population, these only occurred at low frequency (Figs. 2 and 3).

A dendrogram constructed based on genetic distances calculated according to allele frequencies at the *AthF1*, *AthB5* and *AthD6* loci was able to differentiate all the study populations. The population from the Danube Delta showed highest genetic distance, creating a separate branch with a bootstrap index of

100. In comparison, the population from the Kakhovka Reservoir was differentiated from the other three populations with a bootstrap index of 51, while the other three populations were differentiated from each other with genetic distances of 0.5 and 0.6, though the bootstrap indices were low.

Discussion

The results of this study indicate that the big-scale sand smelt from the Gulf of Odesa were most 'distant' from all other study localities, being characterised by their high parasite diversity. Thus, this population (native range) is likely to be the oldest and most stable of those studied.

Compared with other native populations, several common species were absent. For example, the parasitic isopod *Mothocya epimerica* Costa, 1851, is a common parasite of this species in waters of Greece (Leonardos and Trilles 2003), Italy (Bello et al. 1997) and Turkey (Öktener and Sezgin 2000) and

Fig. 2 An UPGMA-tree relating five native big-scale sand smelt populations from different locations based on allele frequencies for three microsatellite loci and Nei's standard genetic distance
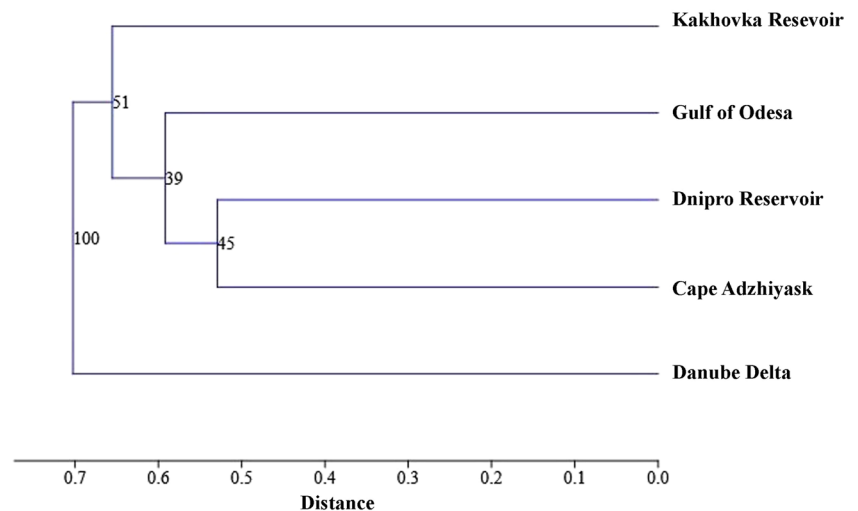
Fig. 3 Principal component analysis (PCA) of big-scale sand smelt sub-populations based on allele frequencies for three microsatellite loci
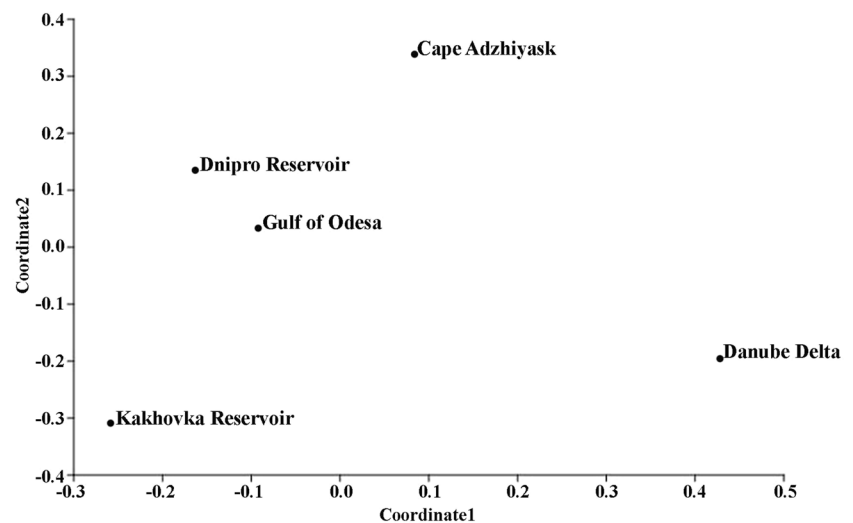
has also been recoded from the Ukrainian coastline (Kvach and Drobiniak 2017); however, it was not recorded at all in the current survey (see Table 2). In all places, it has been found in Ukraine; however, it has only been recorded at low abundance (Kvach and Drobiniak 2017). Likewise, the digenean *Bacciger bacciger*, which has been recorded as common in the Black Sea (Kvach and Drobiniak 2017) and Mediterranean Sea (Culurgioni et al. 2014), occurred only very rarely in the Gulf of Odesa.

Published data on the parasites of translocated sand smelt populations in freshwaters are very rare. In Lakes Massaciuccoli and Trasimeno (Branciari et al. 2016; Guardone et al. 2021) in Italy and Lake Iznik in Turkey (Çolak 2013), translocated sand smelt are known to host larvae of *Eustrongylides* spp. (including *Eustrongylides excisus*). Here, we also report the presence of this parasite in sand smelt from the Danube delta (see Table 2). A second parasite found in introduced sand smelt in the Mediterranean basin, the parasitic copepod *Lernaea cyprinacea* L., 1758, found in the Draa and Ghir basins, Morocco (Clavero et al. 2015), was also not confirmed in the Black Sea basin. In addition to *E. excisus*, three other parasite species were recorded on sand smelt in Lake Iznik, Turkey, i.e. *Bothriocephalus* cf. *acheilognathi*, *Diplostomum* sp. and *Tylodelphys clavata* (Çolak 2013), none of which was confirmed in our study. Note, however, that *B. acheilognathi* and *Diplostomum* sp. are both already known from sand smelt in the northwestern Black Sea (Kvach and Drobiniak 2017).

The parasite community of big-scale sand smelt in its natural range (Danube Delta, Gulf of Odesa, Cape Adzhiyask) was higher than that in its expansion range, which was relatively poor in comparison, being absent (Dnipro Reservoir) or represented by just two sporadic species (Kakhovka Reservoir). These results are in line with the 'enemy release hypothesis', where non-native species escape from parasites in their new habitats, allowing them to establish stable populations (Torchin et al. 2003; Roy et al. 2011). This trend is commonly observed in non-native fish species after invading both freshwater (Sheath et al. 2015; Ondračková et al. 2021) and marine (Merella et al. 2016; Tuttle et al. 2017) ecosystems.

On the other hand, the development of parasite communities in native and expansion ranges does not depend on host population genetics. While parasite community composition in the Gulf of Odesa was most distant from all other localities, that for the Dnipro Reservoir was characterised by an absence of parasites (most distant and newest expansion locality), while only fish from the Danube Delta differed genetically.

Two freshwater parasites were recorded; these being represented by larvae of the nematodes *Eustrongylides* sp. and *Raphidascaris* sp. Both species have already been registered for big-scale sand smelt from the northwestern Black Sea (Kvach and Drobiniak 2017). Interestingly, the presence of freshwater parasites confirms migration of the fish into riverine deltas and then back to the sea. It is most likely that migration/introduction of the species into the Kakhovka Reservoir is responsible for presence of the Mediterranean acanthocephalan *T. exiguus* (see Table 2). In addition to *T. exiguus*, the digenean *T. imbutiformis*, another Atlanto-Mediterranean species, could be used to discriminate fish populations. As in the case of the freshwater nematodes, the species acts as a host for the larvae, the presence of which depends on the density of both definitive and intermediate hosts at the locality. For example, *T. imbutiformis* is a typical marine parasite, which requires *Hydrobia* sp. mud snails as its first intermediate host (see Kvach et al. 2018 and references therein). As their name implies, these mud snails prefer muddy habitats, which dominate the brackish Solonyi Kut Bay in the Danube Delta. The two other localities in the northwestern Black Sea, the Gulf of Odesa and Cape Adzhiyask, are mainly characterised by sandy bottoms, and hence, mud snails are not abundant. Likewise, this marine parasite is absent in the host's expansion range (freshwater reservoirs) due to the lack of a first intermediate host.

The monogenean *Gyrodactylus ginestrae* also acts as a marker-parasite discriminating sand smelt populations as the species, which is specific to the sand smelt, has been described from the Gulf of Odesa and is also known from adjacent open seawaters (Kvach and Drobiniak 2017; Kvach et al. 2019). Recently, it has only been recorded in the Gulf of Odesa, being absent from localities close to the riverine deltas and in host's expansion range (see Table 2). The species' low tolerance to salinity change is typical of the monogeneans, and especially of *Gyrodactylus*, the systematic of which is based on the shape of their osmoregulatory organ (Malmberg 1970; Lebedeva et al. 2021). This is the most likely reason for the smelt's escape from monogeneans in desalinated environments.

Overall, the parasite community of the big-scale sand smelt appears to depend mainly on environmental factors, such as habitat type, salinity and prey composition. Consequently, microsatellite analysis indicated only slight differences between native sand smelt populations and their neolimnetic form in the River Dnipro, with bootstrap data indicating that the population from the Danube Delta was the only population differing genetically from populations at all other localities, presumably as this was oldest and most geographically distant from all other sites. On the other hand, parasite infestation in Danube Delta fish only differed from that in the Gulf of Odesa and at Cape Adzhiyask, showing similarity with the neolimnetics from the River Dnipro. This was most likely due to the influence of freshwater on parasite community formation. Nevertheless, both microsatellite analysis and parasite community species composition both suggest that populations in the River Dnipro reservoirs are not isolated from native populations, as confirmed by the presence of the marine species *T. exiguus* in the Kakhovka

Reservoir and the freshwater *Raphidascaris* sp. in the Gulf of Odesa.

Data availability Not applicable.

Declarations

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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