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ORIGINAL PAPER

Variation in external morphology between the native and invasive populations of the round goby, *Neogobius melanostomus* (Actinopterygii: Gobiidae)

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Abstract

Fish established outside their native range often express various changes in external morphology, which may result in significant variation between individuals inhabiting different regions. In the current study we aim to explore differences in morphology among several populations of the round goby (*Neogobius melanostomus*), an invasive fish in Europe and North America. A total of 753 round gobies were collected from several invasive and native populations, including marine, brackish, freshwater, lake and river ecosystems belonging to the Black Sea, Baltic Sea and Great Lakes watersheds. We analysed 35 metric and meristic characters using ANOVA, discriminant analysis and allometric coefficients. Our results indicate significant differences between most of the populations in characters such as inter orbital distance, eye diameter, head width, second dorsal fin length and depth. River dwelling round gobies had elongated ventral fins compared to those from standing water bodies—a possible adaptation for upstream dispersal in flowing habitats. Most of the morphologic characters expressed positive allometry with significant differences in allometric coefficients between populations. Probably due to their recent invasive history, some of the head characters and allometric models grouped together round gobies from the Great Lakes watershed with those from a newly established population in the Danube tributaries. Similarities between geographically distant round goby populations might reflect common adaptations in external morphology during the course of invasion.

Keywords *Neogobius melanostomus* · Geographic range · Invasive species · Morphological variation · Allometry

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Introduction

Introduction of alien fishes is considered as one of the main causes for loss of aquatic biodiversity, especially in highly sensitive freshwater ecosystems (e.g. Walther et al. 2009; Blackburn et al. 2011; Poulos et al. 2012). Different environmental and geographic factors can lead to variation in body shape, single morphological characteristics or growth patterns within and among invasive populations (Záhorská et al. 2009; Firmat et al. 2012; Polačik et al. 2012; Apostolou et al. 2016; Vila-Gispert et al. 2017). Morphological variability in alien fishes can be associated with differences between native and non-native habitat conditions, predatory regimes, founder effects and others (Langerhans and DeWitt 2004; Firmat et al. 2012; Cerwenka et al. 2014; Vila-Gispert et al. 2017). Both adaptations to the environment and unequal history (clade, genotype, geographic region) play important roles in determining the external morphology of the individuals within an invasive population (Langerhans and DeWitt 2004).

This study is focused on the morphology of the round goby, *Neogobius melanostomus* (Pallas, 1814), a small benthic fish (maximum 250 mm total length) (Sokołowska and Fey 2011) from the Gobiidae family (Perciformes). Its native range includes the Black, Azov, Caspian and Marmara seas and their adjacent wetlands and rivers. Some researchers accept the lower sectors of the Danube River as part of the round gobies native range (Polačik et al. 2012). However, the first reports of *N. melanostomus* in this locality are controversial (Georgiev 1966; Marinov 1978; Karapetkova 1994) and only in the last 20 years there have been numerous records of this species in the lower Danube (Polačik et al. 2008). Therefore, we consider the lower Danube population as invasive (although established early), while round goby inhabiting the Black Sea (and the other Ponto-Caspian localities) are considered as native.

Presently, *N. melanostomus* has invaded a wide geographic range, including salt, brackish, freshwater, standing and flowing water habitats throughout Europe and North America (Corkum et al. 2004). The species is introduced to new regions thought human translocation (e.g. Great Lakes, Baltic Sea) or self-sufficiently (e.g. Danube River and its tributaries) and quickly moves from its initial introduction points to adjacent water bodies (Brownscombe and Fox 2012; Kornis et al. 2017).

The external morphology of *N. melanostomus* from different sections of the Danube River (L'avrinčíková et al. 2005; Polačik et al. 2012; Cerwenka et al. 2014; Jakubčinová et al. 2017) and from the Ponto-Caspian basin (Simonović et al. 2001; Apostolou et al. 2016; Demchenko and Tkachenko 2017; Diripasko and Zabroda 2017) is described in varying degrees of detail. Significant morphological differences were observed between sexes and size classes (Diripasko and Zabroda 2017). Compared to females, male round gobies have larger pre, post and inter orbital distances, smaller eyes, larger jaws, longer and wider heads, higher dorsal fins, longer ventral and pectoral fins as well as a different body shape (Cerwenka et al. 2014; Demchenko and Tkachenko 2017). There are two male morphs corresponding to different reproductive strategies of *N. melanostomus*, which are externally distinguishable based on head width, coloration and size of the urogenital papilla (Marentette et al. 2009; Bleeker et al. 2017; McCallum et al. 2018). In the mid Danube round goby population, different growth patterns (isometric, allometric) have been reported for some body characters as well as cases of ontogenetic shifts in the growth of jaw length, eye diameter, head width, first dorsal fin length and width (L'avrinčíková et al. 2005). Variation in the external morphology of *N. melanostomus* inhabiting different water bodies have been interpreted as an adaptation to specific environmental conditions (Polačik et al. 2012; Apostolou et al. 2016; Demchenko and Tkachenko 2017). However, Cerwenka et al. (2014) observed differences in the external morphology between specimens, regardless of the common habitat. This fact was associated with founder effects (the genetics of the firstly introduced individuals), rather than a collective adaptation toward the environment.

An overall morphological comparison between the non-native and native populations of the round goby has not yet been made. There is a lack of knowledge about the morphology of this species from localities such as the Baltic Sea (Europe), the Laurentian Great Lakes (North America), or the recently invaded Danube River tributaries (Bulgaria). Therefore, we conducted a comparative morphological study of *N. melanostomus* from several localities along its geographic range. Our main objectives are: (1) to analyse round goby morphology and explore possible differences between native and non-native populations; (2) to determine the species' allometric models and compare them across populations; and (3) to discuss possible connections between the external morphology and the invasion success of *N. melanostomus*.

Materials and methods

Study sites and sampling

Fish were collected from one native (Black Sea) and seven invaded locations across Europe and North America (Table 1; Fig. 1). The Black Sea is a confined continental sea, characterised by low salinity, from which round gobies were sampled in the vicinity of Varna Bay (Bulgaria) by local fishermen via trawl and uncovered pound nets. The Danube River is the second largest European river, flowing southeast and emptying into the Black Sea. Individuals of *N. melanostomus* were collected around rkm 432 (Bulgaria) from rip-rap habitats (boulders reinforcing the bank) via backpack electrofishing with pulsed DC (Hans Grassl, 220/440V, 17.8/8.9 A). Pulse frequencies ranged from 45 to 60 pulses. Iskar, Vit and Yantra rivers are tributaries of the lower Danube and represent the current invasion front of *N. melanostomus* in Bulgaria (Dashinov et al. 2018). These sites were also sampled, using a backpack electrofishing unit (SAMUS, DC, 200/350V, 3/12 A) and all collected round gobies were pooled into a single sample. Electrofishing area included different mesohabitats such as pools, riffles and runs; river bottom substrate was dominated by gravel and large pebbles. All captured individuals from the Black Sea, Danube River and its tributaries were treated with an overdose of MS 222 (Sandoz Laboratories) and preserved in 4% formaldehyde. The Szczecin Lagoon and Dabie Lake form part of the Oder River estuary (Baltic Sea, Poland). The bottoms of these water bodies are mostly flat and featureless,

Table 1 List of *Neogobius melanostomus* sampling sites with code, watershed, waterbody, locality, sampling period and number of individuals (*N*, both males and females); the code includes the first letters of the waterbody and watershed number (1—Black Sea; 2—Baltic Sea; 3—Great Lakes)

Site code[a]	Watershed	Waterbody	Geographic coordinates	Sampling period	*N*
BS1	Black Sea	Black Sea coast	N 43.18845 E 27.92673	April to October 2017	72
D1	Black Sea	Danube River	N 44.07137 E 26.68787	April to June 2011	147
DT1	Black Sea	Yantra River	N 43.07596 E 25.55116	April to December 2017	181
		Vit River	N 43.40787 E 24.52179		
		Iskar River	N 43.49874 E 24.24243		
SzL2	Baltic Sea	Szczecin Lagoon	N 53.84842 E 14.53600	July to October 2017	117
DL2	Baltic Sea	Dabie Lake	N 53.51183 E 14.66738	February 2018	46
SCD3	Great Lakes	St. Clair and Detroit rivers	N 42.79061 E 82.47328[b]	August 1993 and September 2013	37
PB3	Great Lakes	Pefferlaw Brook	N 44.33865 E 79.21771[b]	August 2002 and September 2013	51
LO3	Great Lakes	Lake Ontario	N 43.27303 E 79.86846	July 2018	102
			N 43.29991 E 79.84571		
			N 43.26738 E 79.93995		

[a]*BS1* Black Sea, *D1* Danube, *DT1* Danube tributaries, *SzL2* Szczecin Lagoon, *DL2* Dabie Lake, *SCD3* St. Clair and Detroit rivers, *PB3* Pefferlaw Brook, *LO3* Lake Ontario

[b]Specimens from various locations, mid-point of main sampled water bodies provided

composed of fine sediments where pollutants are accumulated (Daniszewski 2014). Sampling was done using fyke nets, set at a depth of 2 to 3 m and the collected round gobies were frozen individually in plastic bags and stored at −20 °C. Subsequently from the Szczecin Lagoon and Dabie Lake only male individuals were caught. Lake Ontario is the smallest of the North American Laurentian Great Lakes (Canada and USA). From this locality fish were collected, using minnow traps, in the vicinity of Hamilton Harbour (on the western tip of Lake Ontario) at three sites which were pooled in a single sample (Table 1). Traps were set at 1 m depth on cobble substrates mixed with mud and sand. All captured round gobies were immediately euthanized by overdosing with a benzocaine solution and placed on ice until they could be photographed (see below). The St. Clair and Detroit rivers flow between Lake Erie and Lake Huron and their bottom is dominated by gravel, cobble and bedrock substrate. Pefferlaw Brook is a small tributary of Lake Simcoe, situated north of Lake Ontario and has gravel and cobble bottom substrate. *N. melanostomus* individuals from these three rivers were fixed in 4% formaldehyde and preserved in 70% ethanol in the collection of the Royal Ontario Museum. The collection is composed of round gobies sampled at different locations, throughout the last 30 years.

Specimens' preservation and processing discrepancies

Round gobies collected from the Black Sea, Danube tributaries, Ontario and Dabie lakes were processed four weeks after sampling or earlier. Individuals from the Szczecin Lagoon were keep frozen for about 5 months and only those from the Danube, St. Clair, Detroit and Pefferlaw rivers were stored several years prior to morphologic measurement. Fish stored for long periods of time shrink, deviating from their real length (Shields and Carlson 1996; Martinez et al. 2013). Different states of the gobies (frozen, fixed or euthanized) might have added additional variability to their morphology, especially in sections like the abdomen, where sinking can be more profound.

Round gobies were placed onto a Styrofoam board next to a measuring gauge with fins spread by needles and photographed in three positions (lateral, ventral and dorsal) using a DSLR camera. Fish from the Danube River,

Fig. 1 Locations of *Neogobius melanostomus* populations from the Black Sea watershed (1), the Baltic Sea watershed (2) and the Great Lakes watershed (3). Each point sampled either from at a single or from multiple nearby sites from Black Sea (BS1), Danube River (D1), Iskar River (DTI1), Vit River (DTV1) and Yantra River (DTY1), Szczecin Lagoon (SzL2), Dabie Lake (DL2), Lake Ontario (LO3), St. Clair and Detroit rivers (SCD3) and Pefferlaw Brook (PB3). The Danube River tributaries Iskar River (DTI1), Vit River (DTV1) and Yantra River (DTY1) were pooled into "Danube tributaries" population (DT1)

Danube tributaries and Black Sea were photographed by the first author, specimens from the Baltic Sea watershed were photographed by the second author and round gobies from Lake Ontario – by the forth author. Individuals from St. Clair, Detroit and Pefferlaw rivers were photographed prior to this paper for the purposes of another morphological analysis, but the photographs were applicable for the present study. All measurements and subsequent analysis were conducted by the first author. The following criteria were introduced to include a photo in the analysis: the specimen should not have any body distortion (acquired during storage or before), such as bent bodies, malformations, damaged fins or sunken bellies.

To remove bias caused by sexual dimorphism (Cerwenka et al. 2014; Demchenko and Tkachenko 2017) males and females were examined separately in the statistical analysis. Sex was identified based on urogenital papilla (Miller 1984). Fish that could not be sexed and/or had a standard length of 30 mm or less were classified as juveniles and were not measured. Male sneaker morphs externally resembling females were excluded when possible. Sneakers were identified after dissection for the Black Sea, Danube and the Danube tributaries samples according to Marentette et al. (2009).

Fish measurements

Morphological measurements included 31 metric characters (8 from the head area, 12 from the body and 11 of the various fins), following L'avrinčíková et al. (2005) (Fig. 2). All measurements were taken in millimeters using Digimizer Version 4.6.1 (MedCalc Software), except for mouth width (JW) which was measured using a digital caliper with accuracy of 0.01 mm. Measurements of fins were taken by drawing a line on the longest ray of each fin. Standard length (SL) was measured from the tip of the snout to the end of the vertebral spine (morphologically near the caudal peduncle at the line connecting the bases of the most anterior rays of the caudal fin) (Fig. 2) (see also Fig. 2 from Hempel and Thiel 2013). Number of rays on the first and second dorsal fin (cD1 and cD2, respectively) and anal fin (cA) as well as number of scales in the mid-lateral series (cLS) were counted. For each fin, all rays were counted without making the distinction between branched and unbranched rays. Since specimens from the St. Clair River, Detroit River and Pefferlaw Brook were photographed prior to this study, we could not measure ventral fin length and width (lack of ventral photos), mouth width and the meristic characters.

Data analysis

All measurements were standardised as percentages of SL. Mean value and standard deviation was calculated for the standartised morphological characters, separately for males and females. For each character, difference in means between populations were assessed using ANOVA after testing for normality and homoscedasticity or non-parametric Kruskal–Wallis ANOVA on ranks if the data was not normally distributed or homoscedastic. Appropriate *post-hoc* tests were applied for all pairwise comparisons by the Holm–Sidak method or the Dunn's test, respectively.

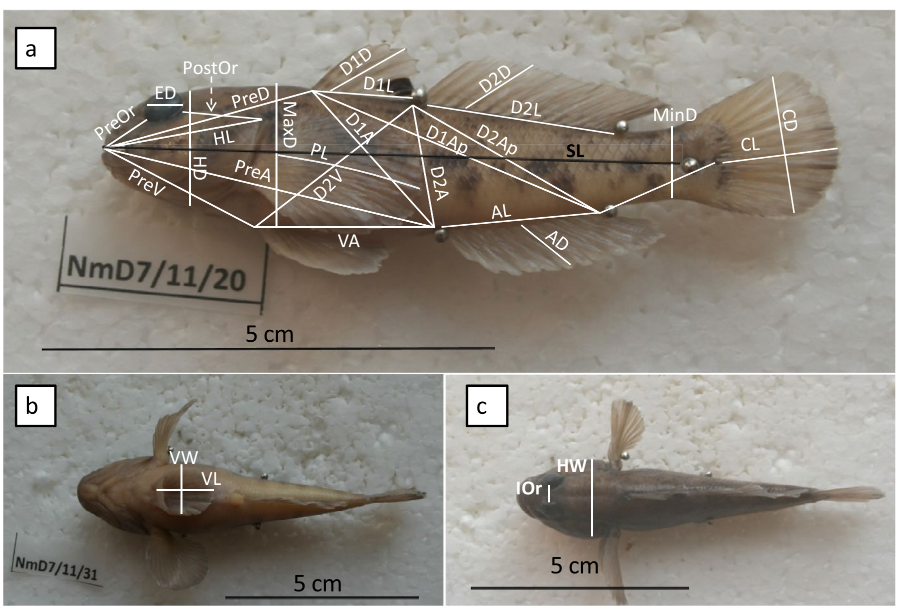

Fig. 2 Photos of *Neogobius melanostomus* from **a** lateral, **b** ventral and **c** dorsal view. Standard length marked in black, metric characters marked in white—anal fin depth and length (AD, AL), caudal fin depth and length (CD, CL), caudal peduncle length (CpL), first dorsal fin base to anal fin base distance (D1A), first dorsal fin to end of anal fin distance (D1Ap) first dorsal fin depth and length (D1D, D1L) second dorsal fin base to anal fin base distance (D2A) second dorsal fin to end of anal fin distance (D2Ap), second dorsal fin base to ventral fin base distance (D2V) second dorsal fin depth and length (D2D, D2L), eye diameter (ED), head depth, length and width (HD, HL, HW), inter orbital distance (IOr), maximal and minimal body depth (MaxD, MinD), pectoral fin length (PL), pre anal distance (PreA), pre dorsal distance (PreD), pre orbital distance (PreOr), pre ventral distance (PreV) post orbital distance (PostOr), ventral anal distance (VA), ventral fin (ventral sucker) length and width (VL, VW)

To differentiate the populations based on their overall morphology a discriminant analysis was performed. All characters were entered one by one using a stepwise procedure where only variables contributing in the lowering of Wilk's lambda are retained in the analysis. Ventral fin length and width, mouth width and meristic characters were not included in this analysis, since they were not measured for all the examined populations. Results were presented as tables of the variables entered in the analysis, cross-validated classification matrices and scattered plots. The Szczecin Lagoon and Dabie Lake samples were excluded from this analysis due to lack of female individuals.

Allometry of the morphological characters in relation to body length (SL) was investigated. After a logarithmic transformation ($\log_{10}$) of the morphological measurements, a linear regression analysis was conducted using a reduced major axis regression (RMA), also known as a standard major axis regression (SMA) (Maie and Schoenfuss 2007; Warton et al. 2006, 2012). The slope of the regression (the allometric coefficient) was used as an indicator of the type of differential growth, considering the 95% confidence intervals, calculated for each coefficient. A value larger than one suggested positive allometry or a higher growth rate of a character in respects to body length. Conversely, if the coefficient was less than one this implied negative allometry or lower growth rate in respects to body length. If the confidence intervals included a value of one, then that character had an isometric relationship with SL—they both had the same (or a very similar) growth. To test for significant changes between the populations' allometric coefficients a common slope test was conducted with the null hypothesis that all slopes of a character are equal. Similarities in allometric models between populations were illustrated by loading the allometric coefficients in a cluster analysis based on Euclidean distances following the unweighted pair-group average method (UPGMA) (Legendre and Legendre 1998).

SigmaPlot 11.0 was used for descriptive statistics and ANOVA. The allometric coefficients and confidence intervals were calculated and common slope analysis was conducted using R studio and the tool package developed by Warton et al. (2012). SPSS 17.0 was used for discriminant analysis and Statistica 7 software was used for the cluster analysis.

Results

Results of the round gobies size range were considered only for sites that were sampled via an unselective method (the Danube River and its tributaries). The Danube tributaries population included relatively small sized individuals (mean SL 54.7 and 60.1 mm for females and males respectively) compared to the Danube River population (mean SL 66.9 and 74.5 mm for females and males respectively) (Suppl. 1 and Suppl. 2).

Significant differences were observed for each morphological character between the examined round goby populations. Head and fin characters showed more prominent differences in mean values compared to characters from the body (posterior of the operculum) (Suppl. 3 and Suppl. 4).

The interorbital distance (IOr) was characteristically longer for round gobies from the Black Sea, Szczecin Lagoon and Dabie Lake (difference in IOr was up to 7.3% SL; $p \leq 0.05$). Conversely IOr was shortest for the Lake Ontario, Pefferlaw Brook and the Danube tributaries round gobies. Eye diameter was larger for the Great Lakes watershed, while in fish from the Black Sea, Szczecin Lagoon and Dabie Lake populations it was smaller. Wider mouths (JW) were observed for round gobies from the Danube River, Szczecin Lagoon and Dabie Lake. Compared to the other *N. melanostomus* individuals, those from Lake Ontario had significantly lower mean values of head width (HW) and head depth (HD).

Round gobies from Szczecin Lagoon and Dabie Lake had longer pre ventral (PreV) and first dorsal fin to anal (D1A) distances, but their ventral anal distance (VA) was shorter than the rest the examined individuals. For Lake Ontario pre anal distance (PreA), first dorsal fin to anal distance (D1A) and VA had the lowest means compared to the rest of the populations (difference was up to 4.6% SL; $p \leq 0.05$).

The width and length of the ventral fin was greater for river populations (Danube River, Danube tributaries) in comparison to those from standing water bodies (Black Sea, Lake Ontario) (differences were up to 6.8% SL; $p \leq 0.05$). Fish from the native population had the shortest ventral suckers. Round gobies from the Great Lakes watershed had longer caudal fins (CL) in comparison to those from the other populations (difference in CL were up to 4.6% SL; $p \leq 0.05$). Conversely, the caudal fins of gobies from the Szczecin Lagoon and Dabie Lake were deeper and shorter (low CL, high CD) compared to the other individuals. Pectoral fins were long for round gobies from standing water bodies (Szczecin Lagoon, Dabie and Ontario lakes) but also for those from Pefferlaw Brook (difference in PL were up to 2.8% SL; $p \leq 0.05$).

Number of the fin rays was relatively uniform between the different round goby populations (Suppl. 5). The first dorsal fin had 6 rays for 91% of all the examined individuals. In the second dorsal fin a mode value of 16 rays was observed. Only for the males from Lake Ontario, this mode was higher (17 rays), while for the Dabie Lake population it was lower (15 rays). The number of scales in the mid lateral series varied more widely for round gobies from the Szczecin Lagoon and Lake Ontario (36–55 scales) in comparison to other populations where scales ranged between 44 and 55 scales.

Table 2 Morphological characters entered in the discriminant model using stepwise method for *Neogobius melanostomus* females with values of Wilks' Lambda and *F* value

Step number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Entered character[a]	IOr	HW	D2L	ED	D2D	D2A	PL	HD	PreV	CpL	CL	HL	PreD	D1D	AD
Wilks' Lambda	0.37	0.22	0.14	0.1	0.08	0.07	0.06	0.05	0.05	0.04	0.04	0.03	0.03	0.03	0.03
F value	90.8	58.9	50.5	42.7	37.9	33.2	29.6	27.5	25.4	23.7	22.3	21.1	19.9	18.9	18.1

[a] *AD* anal fin depth, *CL* caudal fin length, *CpL* caudal peduncle length, *D1D* first dorsal fin depth, *D2A* second dorsal fin base to anal fin base distance, *D2D, D2L* second dorsal fin depth and length, *ED* eye diameter, *HD, HL, HW* head depth, length and width, *IOr* inter orbital distance, *PL* pectoral fin length, *PreD* pre dorsal distance, *PreV* pre ventral distance

Table 3 Morphological characters entered in the discriminant model using stepwise method for *Neogobius melanostomus* males with values of Wilks' Lambda and F value

Step number	1	2	3	4	5	6	7	8	9	10	11	12	13
Entered character[a]	IOr	MinD	D2L	ED	HW	HD	D2D	D2A	VA	MaxD	PL	PreD	CpL
Wilks' Lambda	0.36	0.22	0.15	0.11	0.08	0.06	0.05	0.04	0.03	0.03	0.03	0.02	0.02
F-value	79.7	51.6	40.3	35.1	32	30.7	28.9	26.7	25.1	23.7	22.3	21	19.9

[a] *CpL* caudal peduncle length, *D2A* second dorsal fin base to anal fin base distance, *D2D, D2L* second dorsal fin depth and length, *ED* eye diameter, *HD, HW* head depth and width, *IOr* inter orbital distance, *MaxD, MinD* maximal and minimal depth of the body, *PL* pectoral fin length, *PreD* pre dorsal distance, *VA* ventral anal distance

Table 4 Cross-validated classification matrix for *Neogobius melanostomus* females in percent classified individuals

Sites[a]	BS1	D1	DT1	LO3	SCD3	PB3
BS1	100.0	0	0	0	0	0
D1	0	58.3	41.7	0	0	4.2
DT1	0	4.9	95.1	0	0.7	0
LO3	0	0	7.1	81.0	7.1	4.8
SCD3	0	5.0	20.0	5.0	55.0	15.0
PB3	0	4.2	8.3	12.5	16.7	58.3

[a] *BS1* Black Sea, *D1* Danube River, *DT1* Danube tributaries, *LO3* Lake Ontario, *SCD3* St. Clair and Detroit rivers, *PB3* Pefferlaw Brook

Table 5 Cross-validated classification matrix for *Neogobius melanostomus* males in percent classified individuals

Sites[a]	BS1	D1	DT1	LO3	PB3	SCD3
BS1	91.2	5.9	0	2.9	0	0
D1	8.9	78.6	10.7	0	0	1.8
DT1	1.5	4.6	84.6	0	0	9.2
LO3	0	0	0	94.4	2.8	2.8
PB3	0	0	7.7	7.7	84.6	0
SCD3	0	17.6	29.4	29.4	11.8	11.8

[a] *BS1* Black Sea, *D1* Danube River, *DT1* Danube tributaries, *LO3* Lake Ontario, *SCD3* St. Clair and Detroit rivers, *PB3* Pefferlaw Brook

After the stepwise procedure in the discriminant analysis, 15 morphological characters were included for females, while 13 characters were included for males (Tables 2 and 3). Ten of these characters were common for both sexes, where inter orbital distance, head width and depth, eye diameter and second dorsal fin length and depth, contributed most strongly in the differentiation between populations.

In the discriminant analysis on average 83.7% of the cross-validated round goby females were correctly classified based on their external morphology (Table 4). On average 80.3% of the cross-validated males were correctly classified (Table 5).

Native round gobies (Black Sea) were distinctly separated in the graphs of the canonical discriminant functions. Males from the native range showed only partial overlap with some of the gobies from the other localities, while females showed no overlap (Fig. 3). The rest of the populations were grouped together, but those from the Great Lakes watershed were partially more separated.

Positive allometric growth was observed for most of the examined morphological characters (Suppl. 6 and Suppl. 7). Highest values of the allometric coefficient were recorder for mouth width, ventral fin width and caudal fin depth. Isometric growth was observed for some characters, but predominated only in fish from the Pefferlaw Brook and Dabie Lake populations. Negative allometry was registered in a few cases—for eye diameter, caudal fin length, pre dorsal distance, distances from D1or D2 to the end of the anal fin (D1Ap, D2Ap). According to the common slope test, 19 characters had significantly different allometric coefficients for females and 21 characters had differences in their allometric coefficients for males (Suppl. 6 and Suppl. 7).

Male and female round gobies showed a relatively similar grouping by their allometric models in the cluster analysis (Fig. 4). The Dabie Lake and Szczecin Lagoon populations as well as those from Danube River and Black Sea were separated from the rest and formed distinct clusters. Round gobies from the Danube tributaries and Lake Ontario had similar

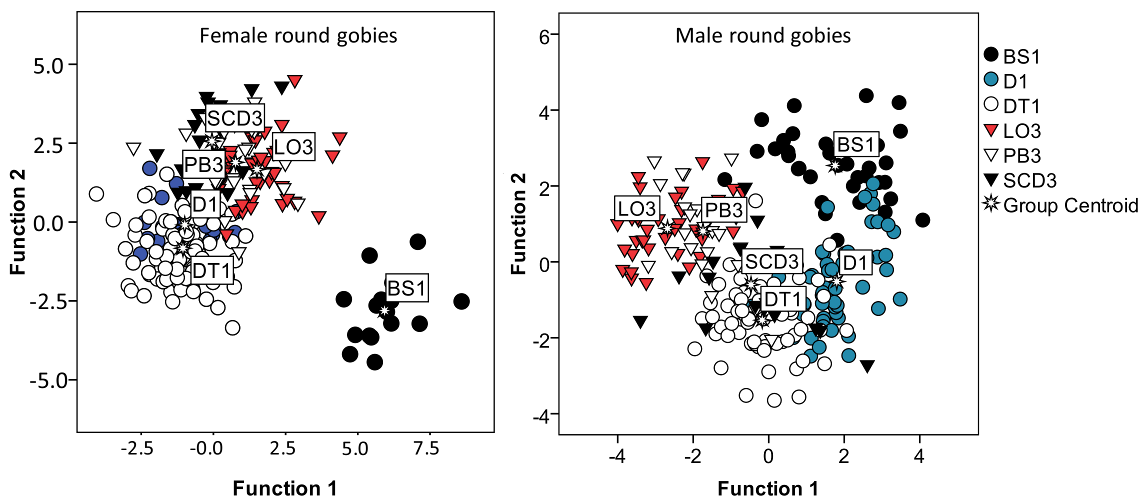

Fig. 3 Scatter plots of the first two canonical discriminant functions for Neogobius melanostomus females (on the left) and males (on the right) from Black Sea (BS1), Danube River (D1), Danube tributaries (DT1), Lake Ontario (LO3), St. Clair and Detroit rivers (SCD3) and Pefferlaw Brook (PB3)

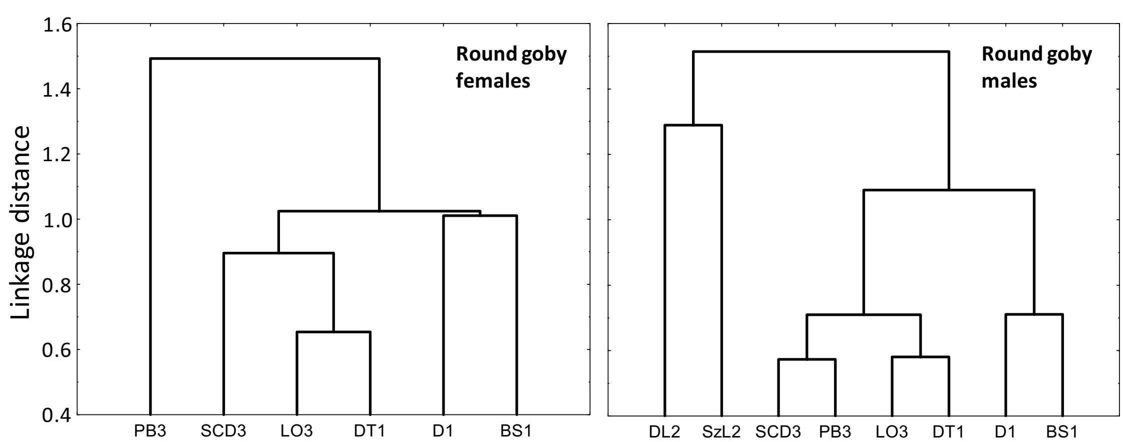

Fig. 4 Dendrogramme based on allometric coefficients for *Neogobius melanostomus* females (on the left) and males (on the right) from Black Sea (BS1), Danube River (D1), Danube tributaries (DT1), Szczecin Lagoon (SzL2), Dabie Lake (DL2), Lake Ontario (LO3), St. Clair and Detroit rivers (SCD3) and Pefferlaw Brook (PB3)

allometric models. The female individuals from Pefferlaw Brook were distinctly separated, while males from the same locality were in a cluster with gobies from the Great Lakes watershed and the Danube tributaries.

Discussion

Differences in the external morphology between the populations of invasive fish may reflect processes of adaptive phenotypic change as well as founder effects and unique population history (Langerhans and DeWitt 2004; Firmat et al. 2012).

A prominent differentiation in external morphology was observed between round gobies from the native (Black Sea) and all invasive populations with a classification power of 100% for females and 91.2% for males. On the other hand, there were differences in the external morphology between populations within the native range of the species. Individuals from the Black Sea had shorter ventral fins, smaller eyes, smaller interorbital distances, and lower depths of the second dorsal fin but wider heads compered to studies of the Azov Sea population (Demchenko and Tkachenko 2017; Diripasko and Zabroda 2017).

The observed allometric similarity between the Black Sea and the lower Danube River populations corresponds to a previously reported low genetic and morphologic diversity of the round goby (Apostolou et al. 2016). The lower Danube is inhabited by one of the oldest non-native population of *N. melanostomus* that originated from the Black Sea, which probably accounts for the observed similarities. In a completely different situation are the round gobies from the Danube tributaries in Bulgaria, who have a very recent history of establishment and form the so-called "invasion front". These fish were distinct in their allometry and morphology from the populations of the Danube River and Black Sea.

Pioneer *N. melanostomus* individuals from both the Danube tributaries and the Trent-Severn Waterway (Great Lakes watershed) (Brownscombe and Fox 2012) are small sized. It has been demonstrated that small-bodied round gobies are better suited for upstream dispersal (Pennuto and Rupprecht 2016). However, contrary to the case of the Danube tributaries the upper section of the Danube River was colonised by large individuals (Brandner et al. 2013, 2018). This is an interesting case of two seemingly contrary invasive strategies within a single drainage basin. Other studies of the Trent River (Great Lakes watershed) also report larger gobies in the upstream invasive front, but not in the downstream edge of the same river (Gutowsky and Fox 2011).

The wide and long ventral sucker of the river dwelling round goby populations suggest an adaption of this fin towards water velocity. Ventral fins are also relatively long in other upstream spreading *N. melanostomus* populations such as those from the mid-section of the Danube River (Simonović et al. 2001; L'avrinčíková et al. 2005). Laboratory analysis reveals that *N. melanostomus* uses its pectoral and ventral fins to maintain a stable position in flowing conditions (Tierney et al. 2011) and elongation of these fins probably gives the round goby a better potential for dispersal.

The round gobies from the Great Lakes watershed had similar head and body characters with those from the lower Danube tributaries. The latter had also similar allometry with the Lake Ontario population in mouth width, inter orbital distance, head width (all with positive allometry) and pre dorsal, pre anal and pre ventral distances (all showing isometry). Similarities between geographically distant invasive populations support a hypothesis for common adaptations in morphology and allometry during the course of the round goby invasion. Apart from this case, similar allometric models were observed for the populations inhabiting common watersheds.

A tendency of shortening in the inter orbital distance was registered in nearly all invasive populations. It would seem that the round gobies from Central Europe and North America have common morphological characters of the eye orbit – large eyes and short inter orbital distance. Lowest values of inter orbital distance were observed in individuals inhabiting relatively small rivers like the Danube tributaries and Pefferlaw Brook. For the mid sections of the Danube River the mean value of this character is even smaller (L'avrinčíková et al. 2005). These findings are not congruent with Polačik et al. (2012), who report that round gobies from low sections of the Danube have larger eyes and smaller interorbital distance then those from mid sections of the same river. However, the reported differences are relatively small (up to 0.8% of SL) compared to results observed in the present study. Round gobies from a freshwater reservoir have larger eyes and smaller interorbital distances then those inhabiting a bay of the Azov Sea (Demchenko and Tkachenko 2017).

Conversely, small eyes and large inter orbital distance were typical for the Black Sea and the two invasive populations of the Baltic watershed. Otherwise, round gobies from the Baltic Sea watershed were very different in their external morphology from the rest of the examined individuals. The habitat conditions in of the Szczecin Lagoon and Dabie Lake are specific, with significant levels of water and sediment pollution (Daniszewski 2014). However, it must be noted that methodological differences in fish fixation and an unbalanced sex ratio in the investigated samples might contribute to the distinction of the Szczecin Lagoon and Dabie Lake individuals. The observed morphological similarities in the eye orbit between latter and the native population might reflect founder effects, since the Baltic round gobies originated from the Black Sea (Brown and Stepien 2008).

The Great Lakes invasive populations were probably formed by individuals from the rivers of Eastern Europe (Dnieper, Dniester and Bug rivers), although the former do not exhibit typical founder effects (Brown and Stepien 2009). Similarities between donor and recipient populations of the round goby are hard to trace back, due to the species' high degree of dispersal and unknown number of introductions (Brown and Stepien 2009; Bronnenhuber et al. 2011). Although morphological differentiation based primarily on founder effects has been reported this requires surveying several sites in a relatively small region (Cerwenka et al. 2014). The current data set does not allow such refined distinctions, but gives a general overview of the round gobies' external morphology across its geographic range.

Our results confirm a global variation in some of the morphologic characters of *N. melanostomus* and are consistent with previously reported morphological differences between populations at a regional scale (Polačik et al. 2012; Cerwenka et al. 2014; Apostolou et al. 2016; Demchenko and Tkachenko 2017). Native and non-native populations were distinct, suggesting a morphological divergence of the round goby during the course of invasion. Individuals, which invaded the Danube tributaries, resemble those from transcontinental invasive populations. These similarities between different, geographically distant non-native populations support the notion that a common morphological adaptation might contribute to the species invasive success.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All used material was handled in compliance with the ethical standards of the involved institutions.

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